

ASSESSMENT OF STRESS AND GROWTH OF THE EEL
"ANGUILLA ANGUILLA" IN A CLOSED
RECIRCULATING AQUACULTURE SYSTEM

Christopher Graham David

A Thesis Submitted for the Degree of PhD
at the
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RECIRCULATING AQUACULTURE SYSTEM

by

Christopher Graham David



Thesis submitted for the degree of
Doctor of Philosophy
in the University of St. Andrews

July 1996

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To My Dear Wife Margaret and Loving

Daughters Jodie and Lauren

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ABSTRACT

1) Closed recirculating intensive aquaculture potentially offers major advantages over existing technologies including reduction in normal production time, reduced water input and output and beneficial environmental effects.

2) The major aim of this study was to produce a basic scientific understanding of the factors that affect intensive recirculating culture of the European eel *Anguilla anguilla* in order to increase efficiency and economic viability of eel aquaculture in the E.U.

3) Unlike some intensively farmed fish such as salmonids little is known of the stress factors affecting optimal growth rates in intensive eel culture. The primary effects of stress are mediated by corticosteroids and catecholamines which may have profound effects on growth, appetite and ion and water balance.

4) Growth rates of the eel *Anguilla anguilla* were investigated in closed water recirculating systems utilising fresh water or saline water (12 ppt) at 23°C. Eels were initially graded into two similar populations consisting of three categories, small (12g), medium (24g) and large (48g) based on initial growth rates.

5) During a 300 day period the medium and large group's growth rates were significantly greater in 12 ppt saline water than in fresh water, although for the small fish group there was no such difference. Stocking densities were maintained at commercial levels of approximately 30-100 kg/m³.

6) Plasma cortisol concentrations increased throughout the growth period in both fresh and saline water, although there were no significant differences between the two groups during the experiment. Metabolic clearance rates of cortisol were however consistently higher in saline water fish.

7) Both groups showed an increase in plasma glucose concentration throughout the experiment. However there were no significant differences between fresh water and saline water fish for plasma concentrations of glucose, free fatty acids or lactate.

8) Eels held at stocking densities of 130 kg/m³ continued to grow in the saline water whereas the control fish in fresh water ceased growing. The results suggest that maintaining water salinity at 12 ppt in closed recirculating aquaculture systems produces increased growth rates and possibly increased efficiency of food conversion.

9) In response to acute grading stress, plasma osmolality and glucose concentrations were elevated in both fresh and salt water groups 20 minutes after grading but returned to pre-grading values within 90 minutes. Plasma cortisol concentrations were

elevated after 20 and 40 minutes in saline water but returned to control values after 90 minutes. In fresh water fish, plasma cortisol concentrations were elevated after 20 minutes and remained elevated throughout the experiment.

10) Acute netting stress (tank transfer) resulted in a transient increase in plasma osmolality within 20 minutes after net transfer. Plasma cortisol concentrations were significantly elevated after 20 minutes in saline water but returned to control values after 60 minutes. In fresh water fish, plasma cortisol concentrations were elevated throughout the 90 minute period monitored after net transfer.

11) In both cases of acute stress (netting and grading), plasma catecholamines were elevated within a five minute period after the stressor was applied.

This study has developed techniques to assess both long-term and short-term stress in eels and has optimised the environmental conditions leading to improved growth rates. Improvements in the performance of recirculating aquaculture for on-growing eels have been demonstrated and suggestions for future possible improvements as a way forward in commercial aquaculture have been suggested. These factors will, hopefully, lead to increased economic efficiency and increased profits in eel aquaculture within the E.U.

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ABBREVIATIONS

A I	Angiotensin I
A II	Angiotensin II
ACTH	Adrenocorticotrophin
ANP	Atrial Natriuretic Peptide
AVT	Arginine Vasotocin
BKD	Bacterial Kidney Disease
BNP	Brain Natriuretic Peptide
BOC	British Oxygen Company Ltd.
BOD	Biological Oxygen Demand
CA	Catecholamine
CNP	C-Type Natriuretic Peptide
CRF	Corticotrophic Releasing Factor
DIP	Dissolved Inorganic Phosphate
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
DOP	Dissolved Organic Phosphate
EU	European Union
GAS	General Adaption Syndrome
GFR	Glomerular Filtration Rate
GH	Growth Hormone
HK	Highly Specific Hexokinase
HPI	Hypothalamus-Pituitary-Interrenal
MCR	Metabolic Clearance Rate
MSH	Melanophore Stimulating Hormone
MS222	Ethyl m-aminobenzoate Methane Sulphonate
NLT	Nucleus Lateralis Tuberis
PFFA	Plasma Free Fatty Acid

POP	Particulate Organic Phosphate
ppt	Parts Per Thousand
PRL	Prolactin
RAS	Renin-Angiotensin System
RBC	Red Blood Cell (Erythrocyte)
rpm	Revolutions per Minute
SS	Suspended Solids
TOC	Total Organic Carbon
U I	Urotensin I
U II	Urotensin II
UVc	Ultra Violet Light (wavelength 260 nm)
VIP	Vasoactive Intestinal Peptide
VNP	Venticular Natriuretic Peptide

CHAPTER 1

General Introduction

CHAPTER 1
GENERAL INTRODUCTION

1.1 AQUACULTURE:- AN HISTORICAL PERSPECTIVE

The origin of aquaculture is difficult to determine precisely as it has evolved as a means of food production in parallel with man's development over many centuries (see table 1:1). Four major taxonomic groups are cultured by man; algae, molluscs, crustacea and fish. Higher vertebrates are also cultured, although not in large quantities, these include frogs (amphibia) for food and laboratory use, alligators (reptilia) for their skin and meat and turtles, a renowned eating delicacy. However, for economic, technical and cultural reasons out of over 20,000 fish species less than 100 are farmed (Barnabe, 1990)

Early aquaculture employed extensive farming techniques. The earliest recorded example of aquaculture consists of an Egyptian bas-relief painted on the tomb of Aktihetep, circa 2500 BC, which appears to depict men removing tilapia from a stocked pond (Landau, 1992). Around 2000 BC, wild populations of carp were encouraged to grow in drainable ponds in the Indo-Pacific regions especially China and the Bible refers to "sluices and ponds for fish " in the book of Isaiah, written in approximately 700 B.C. These records indicate that man has been cultivating fish species for over 2,000 years.

TABLE 1:1

Historical Milestones in Aquaculture

Modified After Ruck (1968)

HISTORICAL MILESTONES IN AQUACULTURE

- 2000 BC Carp cultivated in China; fishing and rearing of tilapia is shown on wall paintings in Egypt.
- 200 BC Oyster culture by the Romans.
- 1200 AD rearing of common carp by monks in Central Europe.
- 1235 Mussel culture developed in France.
- 1500 Beginning of extensive prawn culture as a second crop on paddy fields in South East Asia.
- 1673 Discovery in Japan that oyster spat will settle on upright bamboo stakes anchored to the sea bottom (oyster culture in Japan has rapidly increased over the last fifty years).
- 1741 First trout hatchery in Germany; first attempt to strip eggs from mature broodstock and hatch trout artificially.
- 1820 Start of commercial eel farming in Japan.
- 1857 First hatchery for the propagation of Pacific salmon in Canada.
- 1934 Artificially induced spawning with carp by treating with sexual hormones, discovered by a Brazilian biologist.
- 1934 First success in spawning and partial rearing of kuruma shrimp by Hudinaga in Japan.
- 1963+ Development of the catfish industry in the USA
- 1968+ Industrialised sea based salmon and trout production in Northern Europe.
- 1976+ Boom in shrimp culture—in Taiwan with Tiger shrimp—in Ecuador with white leg shrimp

1985+ Beginning of commercial farming of sea bream and sea bass in the Mediterranean.

Early attempts at aquaculture generally consisted of stock management rather than farming and were limited to fresh water species since the marine environment was considerably more hostile. The Romans reared fish in ponds as evidenced by the circular ponds still visible at Lago di Paola, Sabaudia, Lazio, Italy (Huet, 1975). According to accounts of Pliny, a Roman historian, the Romans cultured oysters in the sea and Aristotle mentions the Greeks culturing oysters in around the same era. These accounts refer to the first attempts at mariculture. Monasteries in the middle ages developed the culture of carp for food by stocking ponds and castle moats (Shepherd and Bromage, 1992). This practice died out as food quality improved and fresh water fish became less popular than sea water fish due to what was then considered to be their inferior "muddy" taste. Fresh water fish are, however, still popular in central and eastern Europe and are produced on increasingly commercial scales as aquaculture technology improves.

For fresh water fish, the most universally favoured species are the various groups of trout. It was during 1741 that the first trout hatchery was established in Germany (Ruck, 1989) and eggs from mature broodstock were stripped and fertilised. In 1856 in Russia the "dry method" of fertilization (where eggs are fertilised by milt in a container containing the eggs and no water) was discovered by Vrassky. Richard Nettle successfully incubated and hatched brook trout in Canada in 1857 and in 1864, Seth Green increased fertility in trout eggs by 50% (Landau, 1992). The rainbow (steel head) trout farming industry began at

the beginning of the twentieth century (Huet 1975). It was not until the 1940s that successful enterprises outnumbered failures and this was primarily due to increased knowledge of dietary requirements, physiological requirements, disease treatments and behaviour. These developments lead to the massive global trout farming industry of today and, just as importantly, paved the way for the salmon farming industry that was rapidly established in Scotland and Norway in the 1960s and which has now spread to other temperate regions of the world such as Canada, USA, New Zealand and Chile (see below). Fresh water fish farming has only been of any significance in Africa since the Second World War with the discovery of the suitability of tilapias, such as *Sarotherodon mossambicus*, as a farmed fish. In the 1960s fresh water fish farming had an impact in the USA when catfish farming became a boom industry.

In Japan, eels have been in great demand for centuries. They are cooked in different styles to produce delicacies such as kabayaki, shirakaki, nagakaki and kuchi. Different methods of eel farming have evolved to meet the huge demand, from outdoor ponds to closed systems using sea water or fresh water. The commonest method used is fresh water outdoor ponds. In this type of aquaculture, four main configurations are used:

- 1) the outdoor pond
- 2) the basic greenhouse pond
- 3) the pond and sedimentation unit
- 4) the pond and the biofiltration unit (Gousset, 1992)

The eel has always been in great demand in European countries such as Denmark and Holland where small 150g. whole smoked eels are popular and Germany where larger narrow headed silver eels 350g. are preferred (Tesch, 1977). The eel industry in Europe relied on catches from the wild fishery but this declined due to pollution and land reclamation. In response, the most intensive form of aquaculture using heated water recirculation systems was developed.

The development of marine fish farming probably started in Polynesia before AD 1000 by trapping young milkfish in ponds at high tide and then rearing them to maturity. This practice was transferred to Hawaii where fish ponds dating back to 1000 AD can still be seen (Landau, 1992). Similar methods are used today especially in Taiwan. Marine fish farming in the area has become very intensive with the advent of yellow tail (*Seriola lalandi*) farming in cages in the 1960s. In Europe the most significant development in marine farming was the mastering of the complete life cycle of the Atlantic salmon *Salmo salar* which, being anadromous, lays eggs and spends its juvenile period in fresh water and then migrates to the sea. As early as 1912 there was an attempt to grow rainbow trout in the sea in Norway but it was not until the fifties that the Vik brothers at Sykkylven proved the sea cage method to be commercially viable (Edwards, 1978). This method of farming expanded into the sixties and seventies and led the way to the rearing of salmon in sea cages, after juvenile fish had been reared in fresh water under conditions similar to those used for trout fingerlings.

1.2 PROBLEMS ENCOUNTERED BY THE SCOTTISH SALMON FARMING INDUSTRY

The aquaculture industry in Scotland depends largely upon trout and salmon farming. The overall aim of this project was to develop a different type of culture system of a new species, and to appreciate the rationale of the study it is necessary to consider the current status and problems facing the Scottish industry.

Economies in remote areas have always been fragile and the Highland and Islands of Scotland have certainly been no exception. The development and establishment of the salmon farming industry were welcomed by all and generously supported by Government bodies such as the Highland and Islands Development Board. Presently the salmon industry employs over 6,000 people directly and indirectly in remote areas producing fish valued at over £140 million per annum.

During the rapid growth of the Scottish salmon farming industry in the 1960s-1980s the commercial value increased rapidly and fragile rural economies were bolstered to a point of reliance on this new industry. In the late 1980s the "bubble economy" burst with the industry overproducing and prices crashing from £5.50/kg in the early 1980s to as low as £2.20/kg in the early 1990s. Furthermore the Norwegian government were subsidising their production, further adding to the economic problems faced by the Scottish industry. In addition to overproduction the industry

also came under the scrutiny of the environmental lobby. No longer were fish cages considered to be the saviour of remote economies but were now regarded as potential polluters of the aquatic environment (Pocklington *et al.*, 1994.).

Commonly lodged environmental objections are:

Sea lice - Marine fish farms began to be plagued by sea lice and acted as reservoirs of sea lice production. The report published by the Sea Trout Task Force, 1994, alleged that sea lice originating from salmon farms were involved in the drastic reduction of natural sea trout populations on the West Coasts of Ireland and Scotland.

Antifouling - Antifouling paints used to deter marine growth on sea cage nets were toxic, containing copper or tributyl tin (TBT), and interfered with the gender of certain gastropods. The use of these has since been made illegal.

Organophosphates - Nuvan (Ciba- Geigy) used to combat sea lice was cited by the environmentalists as causing untold damage to shellfish larvae.

Antibiotics - Antibiotics, some used to treat human pathogens, were employed and often misused leading to resistant strains of fish bacteria. Some strains of *Aeromonas salmonicida* have been isolated which now produce antibiotic neutralising enzymes capable of destroying the effects of new antibiotics.

Noxious effluent - River purification boards became increasingly aware of problems of hypernutrification especially due to the supply of limiting nutrients to the ecosystem (i.e. phosphorous in fresh water and nitrates in salt water) resulting in phytoplankton blooms. Levels of biological oxygen demand (BOD), total organic carbon (TOC), suspended solids (SS), ammonium, nitrogen and total nitrogen increased in rivers into which fish farms discharged and fresh water lochs in which cages were sited. Sea beds were suffering due to azoic layers forming from uneaten food and faecal solids falling from the cages. Cage fish farming production of 50 tonnes per annum (relatively small by modern standards) is reported to generate organic waste that corresponds to discharges of a sewage treatment plant serving a town of 7,000 inhabitants (Hakanson *et al.*, 1988). Effluent from river based fish farms is constantly accused of causing wild fish stocks to dwindle.

Aesthetics - the sharp outlines of the regimented rows of fish cages contrasted with the soft outlines of the magnificent West Coast Scottish scenery and the farms were described as "eyesores" and in conflict with the well established, and economically important, tourist industry.

Genetic pollution - Farmed fish were genetically selected to optimise performance under cultured conditions; escapees were blamed for polluting wild gene pools and possibly out-competing existing stocks. As a result of storm damage it is possible for thousands of salmon to escape through ruptured nets and cross

breed with wild populations.

Further problems developed for the industry which were mainly self inflicted some of which are listed below:

Monoculture - During the development of salmon farming in the 1980s millions of pounds were invested in a single species, salmon. The subsequent economic decline demonstrated the problems of mono culture and the industry has shown no capability to diversify into the production of new species. This is widely recognised as a limitation for the expansion of aquaculture in Scotland.

Predators - Seals, cormorants, herons and humans are all potential problems especially in established farms when the predator knows where a "free meal" can be found. Some predators will cause immense damage in one visit killing fish they cannot grasp. Due to the very nature of fish farms they are located in remote places and, especially cage farms, are prime targets for theft by organised criminals.

Diseases - Diseases, many of them resistant to treatment, were spreading from farm to farm either by tides from one sea loch to another or by wild fish becoming infected and carrying the infection to another farm. Examples are numerous but typically include furunculosis, vibriosis and bacterial kidney disease (BKD) (Roberts, 1989).

Sea lice resistance - Sea lice were becoming resistant to treatments and could not be eradicated as they reinfected the same farm and spread from one farm to another.

Droughts - With the recent hot summers and associated low rain fall many hatcheries on the West Coast of Scotland have lost stock due to lack of water. One pilot scheme lost their complete stock of arctic char due to increased temperatures as there was too little throughput of water to maintain low temperatures (Kelly, R.- M.D. Strathaird Farms Ltd. pers. comm.). Many fish succumb to high temperatures due to the lack of dissolved oxygen (see section 2.1.2.g, chapter 2).

Plankton blooms - Plankton blooms, depending on the species, can be either toxic, mechanically abrasive to the gills or oxygen removing at night. Such blooms can develop under calm sunny conditions and completely destroy whole fish farms containing hundreds of tonnes of fish.

New diseases - Seemingly untreatable diseases appeared, e.g. pancreas disease, thought to be viral in origin (McVicar, 1988). In this condition the exocrine pancreas cells disappear completely leaving completely naked pyloric caecae and the fish loses body mass and often dies. Hitra disease was a condition responsible for many losses in the Norwegian salmon farming industry first noticed off the island of Hitra. It is thought to be caused by the bacterium *Vibrio salmonicida* (Roberts, 1989).

Opportunist diseases - Many diseases are seasonal for example, the causative agent of vibriosis, *Vibrio anguillarum*, is present all year round in the environment and only becomes infective in the high temperatures of the summer and, as such, cannot be eradicated from the system.

Environmental pollution - Fish farms using water from an external source are at the mercy of the contents of that water. Particularly susceptible are fresh water farms extracting water from rivers. Rivers by their very nature collect water from large catchment areas and also any pollutants with often catastrophic effects. Common causes of fish deaths in thisway are fertilizers and pesticides from farming or forestry activities within the catchment area. These pollutants can either be directly toxic to the fish or, by hypernutrification, can cause bacterial or algal blooms which act as described above and may decimate fish stocks.

Storms - Storms on the West Coast of Scotland are quite common and can rupture nets and destroy cages and entire farms. In marine aquaculture oil pollution from massive oil tankers shipwrecked by storms, such as the disastrous grounding of the Braer in Shetland in 1995, can destroy fish stocks.

Scarcity of sites - Cage farms have specific requirements including shelter, proximity to shore bases (owned or leased by the owner of the farm) which are well serviced by roads, electricity, domestic water and clean pollution-free water.

Also permission has to be sought from various bodies such as:

- 1) The Crown Estate Commissioners who lease the sea bed
- 2) The Department of Transport who assess the degree of hazard to shipping
- 3) The relevant river purification board who assess the environmental impact of the farm
- 4) The local Planning Authority will be automatically notified by the Crown Estate Commission for comments on the shore base facilities

Other parties who may have an interest in the area proposed for the fish farm are:

- 1) The Health and Safety Executive
- 2) Fisherman's Associations
- 3) The Scottish Natural Heritage
- 4) The Countryside Commission
- 5) The Royal Yachting Association
- 6) The Scottish Salmon Growers Association.

Environmental impact assessments (EIAs) are now essential prerequisites to aquaculture in most countries (Pillay, 1992). The list of legal requirements is now very comprehensive and as a result very few new farms are established today. Fresh water cage farms and river based farms are under similar legislative pressure and new ones are rare.

1.3 FUTURE DEVELOPMENT OF AQUACULTURE

IN SCOTLAND

Despite the internal and external problems faced by the Scottish fish farming industry, as outlined in the previous section (1.2), the industry is presently surviving although many smaller producers have either ceased trading or been bought by large multi-national companies. Survival is mainly the result of increased technology, more advanced husbandry techniques and a more sympathetic approach to environmental issues. It is difficult, however, to predict a growth of current farming practices in the future due to the reasons previously outlined (see section 1.2). It is widely recognised that the industry must look for alternative methods, and species, if it is to expand in the future. Practically all the problems referred to relate to the fish farms being in an open environment which is largely beyond the control of the farm manager. All the fish farmer can hope to achieve is to live with the external factors and move with them or insure against them at considerable cost.

If the fish farm could be isolated from external effects the farm manager would, in theory, be in complete control of the production process. In particular by recirculating the fish farm water, the manager would no longer be at the whim of the climatic and seasonal influences on water quality. The benefits of recirculation are immense and, with reference to the previous section, are as follows:

Sea lice - In an enclosed system any ectoparasite such as sea lice once eradicated would not return.

Antifouling - Toxic paints are not used in closed systems as they would effect the fish. Fouling from aquatic growth can be controlled by light regimes and other treatments such as ultra violet light.

Antibiotics - Any orally administered drugs are kept within the system and biodegraded by the biofilter.

Noxious effluent - In most conventional systems there is a small routine daily water exchange, dependant on system design (Liao and Mayo, 1974). Water exchange of 10% per day is normal with 3-5% being considered good (Willoughby, 1995). Virtually all systems remove solids (waste food and faeces). Therefore as the effluent has been through the biofiltration process many times it is discharged as the end product of the nitrification process (i.e. nitrate see section 2.1.3b). The effluent water has a very low BOD and is non toxic to the surrounding environment. The solids removed can be stored in tanks and treated further by conventional sewage treatment techniques. It is obvious that the situation described is far superior to untreated effluent discharge of conventional open systems.

Aesthetics - One of the advantages of recirculating systems is that if 100% recycling is achieved they can be situated on sites reserved for industrial purposes using artificial sea water

located hundreds of miles from the sea. The unit could be housed in a shed on a site set aside by the local Planning Authority for industrial use and not in an environmentally sensitive area.

Genetic pollution - Escape would be most unlikely if not impossible and there would be no chance of an escaped fish entering the natural ecosystem.

Monoculture - One of the main advantages of controlling the environment in which the fish live is that optimum conditions for fish culture can be supplied. Many fish will live under the same environmental conditions and a polyculture system can be operated. Farms within the E.U. culture eels, sturgeon, catfish, tilapia, trout and carp in the same system. Species farmed can therefore change according to the market forces.

Predators - As the system is housed in a secure building natural predators are totally excluded. Theft will be minimised as the farm will not be in an isolated location and alarm systems can be installed which deter theft and can be responded to rapidly in the event of intrusion.

Diseases - By having a stringent quarantine procedure the chances of introducing diseased stock to the farm are minimised. Any influent water can be disinfected with ultra violet light (UVC) or ozone to destroy pathogens entering from outside. Should disease break out it must be treated immediately, as these farms operate at high temperatures, however once under control disease

can be eradicated rapidly from the system.

Droughts - If the farm is 100% recycling then the only influent water is to compensate for evaporative loss and requirements are minimal (less than 5% per week in this study). Even systems utilising water exchanges of 10% per day have a water requirement which is small in comparison to through-flow systems.

Plankton blooms - Plankton blooms, a common problem in sea water, are unlikely in fresh water although silicious diatoms can cause mechanical damage to gills on some river based farms. Should the make-up water supply become a problem due to planktonic inclusions, the farm manager would not extract any water from this source until the problem has subsided.

Environmental pollution - Should the make-up water from which the recirculating farm is extracting become polluted, the farm will simply stop extracting from that source until the pollutant has been removed.

Storms - Risk of storm damage will be drastically reduced. Structural damage to the building would occur only under the most extreme conditions.

Scarcity of sites - With sites discharging into the environment becoming scarce the, advantages of a fish farm that can be located almost anywhere are inherently apparent.

Considering the evidence outlined above one may question why the aquaculture industry has not embraced recirculation technology as the answer to its many problems especially as the technology exists and is reliable (Willoughby, 1995). Common problems preventing the universal adoption of recirculating aquaculture systems are:

Capital costs - The most commonly faced problem is that of the cost of capital equipment which is high relative to traditional intensive systems. This is true and unavoidable but with Government and E.U. financial backing this could be alleviated in order to establish this type of aquaculture industry.

Complexity - Another objection is the relative complexity of the system to run compared to the more extensive cage based systems. This is true but can be overcome by extra training and vigilance.

Running costs - Running costs are undoubtedly higher with the additional cost of liquid oxygen, electricity for running pumps and compressors and the cost of heating the water. However, it is generally argued that decreased production time more than compensates for this.

One of the aims of this study was to improve the techniques used in closed recirculating aquaculture system for a species new to Scottish aquaculture, the European eel *Anguilla anguilla*. In order to appreciate the development of eel aquaculture and its current status, it is necessary to understand the history and

background to the supply and demand for eel in different regions of the world. These aspects will be discussed in the following section.

1.4 EEL AQUACULTURE

1.4.1 EEL CONSUMPTION

Anguillid eels are one of the most widely distributed class of teleost fish in the world, occupying a very wide range of temperature and salinity habitats. They are a common food source in most countries although in some cultures, including Britain, the popularity of eels is not universal, perhaps due to their semblance to snakes. There have been numerous archaeological finds which indicate, that amongst other fish, the eel has been part of man's diet for centuries. Details of the Danish eel fishery dates back to 1885 (Tesch, 1977) and the Italian fishery to 1798 (Bellini, 1907). In Britain the fishery has always been small and localized in the south, supplying the highly specialized jellied eel market centred around London.

The world catch of eels was relatively stable between 1938 and 1973 at between 18,000 and 51,000 tonnes (Yearbook of Fishery Statistics FAO) the peaks and troughs being attributable to market demands and periods of war. However, the catches in recent years have been greatly reduced for a variety of reasons including environmental pollution and attack by the pathogen *Anguillicola* which effects swim bladder function (Knosche, 1991). Huet (1975) explains the reduction in the wild eel catch, at least in part, by the lack of elvers swimming upstream on their migration from the sea. Possible reasons for the decline in elvers are pollution, over fishing and physical obstruction such

as dams. These reductions in catches, together with demand exceeding the supply, lead Knosche (1991) to the conclusion that aquaculture is the only possible solution to this deficit from the wild catch. It is estimated that the world production of eels in 1992 was 120,000 tonnes, 90% of which was of farmed origin (Goussett, 1992).

1.4.2. EEL CULTURE IN EUROPE

Eel culture dates back over 2,000 years when eels were kept in ponds in Macedonia and regarded as culinary delicacies (Eales, 1968). Large lagoons at Comacchio, Italy, were well established in the eighteenth century in an early attempt to culture eels by trapping migrating eels ascending rivers to stock lagoons. A similar but more advanced method of brackish ponds was also established in the eighteenth century at Arcachon near Bordeaux where ascending migrating eels were utilised together with stocks from elsewhere (Le Dantec, 1953 in Tesch, 1977). The first attempts to augment the natural diet by supplying additional food were made in 1889 (Gobin, 1889 in Tesch, 1977).

Currently little extensive farming of eels is conducted in Northern Europe due to the low environmental temperatures but there is a considerable amount of lagoonal production in Mediterranean regions, the Rhone Delta and the Vallicoltura systems in the Italian Adriatic Sea. Since the 1950s semi intensive activity has increased in these areas with parts of

lagoons being sectioned off and aerated and/or supplied with flowing water. Intensive farming of eels has developed in Northern Europe over the last 30 years mainly in Holland, Denmark and Germany where high technology recirculation systems have been developed with artificially heated water and the supply of high performance pelleted diets (see section 2.2.2.a chapter 2).

1.4.3 EEL CULTURE IN JAPAN

To analyze the establishment of eel farming one has to study the Far Eastern countries, especially Japan, where eel has been eaten in large quantities for centuries. Reports of kabayaki (a typical eel dish) exist as early as 1286 (Matsui, 1972). During 1988, 93,666 tonnes of eel was consumed in Japan with 39,558 tonnes being produced using aquaculture techniques (Gousset, 1992). This shows Japan to be the largest single consumer in the world. However, in production terms, Japan is second to Taiwan which has more favourable climatic conditions for semi intensive culture. Eel farming started to develop as a monoculture at the turn of the century with eels reared in ponds of less than a hectare. 1965 saw the "eel boom" with a large increase in the area utilised for eel ponds corresponding to the limiting of paddy field acreage by the Government. However, by 1968 the industry was severely affected by gill rot, saprolegniosis and branchionephritis and faced severe financial problems. This was compounded by Taiwan banning the export of glass eels (the starting point of culture) to promote its own industry. 1968-1972

saw Japanese production drop from 23,640 to 13,855 tons. This lead to the modern day technique of reducing the size of ponds to enable them to be covered by a green house to heat the water cheaply. Increasing areas of land unsuitable for vegetable production were used for eel culture until today it is once again a well established industry. It is perhaps important to note that the surface water quality in Japan is deteriorating which may force some degree of treatment of the pond water, possibly by biofiltration, in order to maintain expansion of the current industry (Gousset, 1992).

1.4.4 THE LIFE CYCLE OF THE EEL

In order to understand the culturing of the Anguillid eel, an understanding of its life cycle is essential. Life cycles of all the fresh water eel species are similar but occur in different geographical locations. For this reason the life cycle of the European eel *Anguilla anguilla* (used in this study) is described.

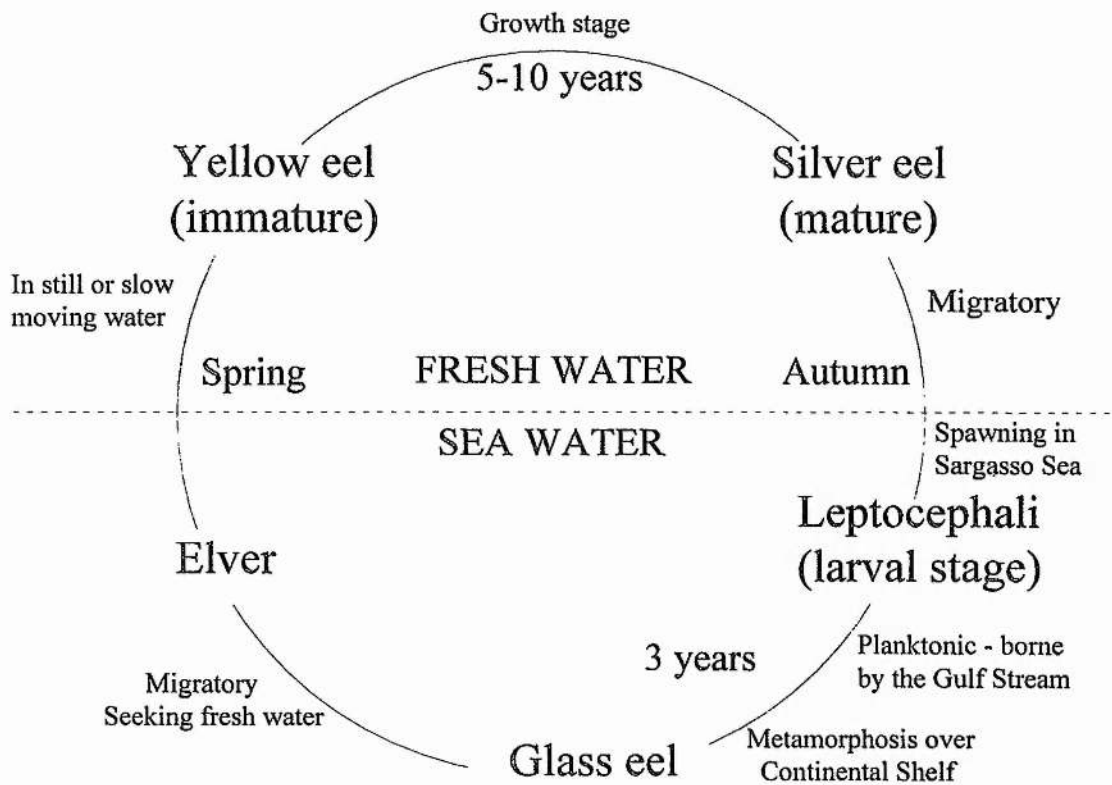
A rather unusual feature of the eel is that it is catadromous in that it spends the vast majority of its life in fresh water, migrating to the sea to breed after which it dies (Figure 1:1). This is the opposite to the anadromous nature of the salmon and some species of trout which live and grow in the sea and migrate into fresh water river systems to breed.

FIGURE 1:1

The Life Cycle of The European
Eel *Anguilla anguilla*

LIFE CYCLE OF EUROPEAN EEL

Anguilla anguilla



During its growth stage, the eel lives in still waters, in lakes or slow moving rivers, preferring muddy substrata where it leads a somewhat sedentary life and can hibernate in the cold winter months. The eel will be, at this stage, a brown/yellow colour. After approximately 5-10 years the eel changes physiologically in a manner not dissimilar to a salmon fry becoming a smolt. Morphologically, the skin colour changes to silver, a camouflage more in keeping for a pelagic life in the sea; the body becomes more streamlined and the eyes become enlarged. The fat content of the body at this stage is high. These features make migration of several thousands of miles possible. Usually on a full moon (possibly to give some light) in Autumn and when the river is in spate, the eels will migrate from the still waters of lakes, ponds and slow stretches of rivers and head downstream. This is when the traditional eel fishery is exploited with large densities of silver eels all migrating and fyke nets are put across their paths to capture them. The eels are highly prized for their appearance and high fat content which is especially suited to the smoking industry. Eels in isolated waters such as ponds will migrate to the river by slithering overland through vegetation. This is achieved by sealing the operculum with mucus and respiration occurs through the skin. Once in the river the mature eels swim downstream to the sea.

What happens to the eels in the sea is an age old mystery. Whether they shoal, swim on the bottom or in top layers of the water and the method of navigation all remain part of the mystery. Eel traps, off the German coast of the Baltic, were

rarely set at below 10 metres and failed to catch eels; this suggested that the eel is not swimming in the surface layer. Eels have been found in the stomachs of pelagic fish near the Sargasso sea and therefore it was considered that they swim in the pelagic layer. It was the classical pioneering work of Schmidt (1923) that pinpointed the breeding ground of the eel to be the Sargasso sea (a sea of similar area to Wales situated between Bermuda and the mainland). Schmidt was able to pinpoint the location by following back the number of larval forms of the eel (leptocephalus) caught in the area until he concluded that the higher the density of leptocephali, the nearer was the source or breeding grounds. It was assumed that the location of the leptocephalus occurred near the spawning grounds.

The eel is generally considered, therefore, to spawn in the Sargasso sea and the leptocephalus larval stage then drifts north on the Gulf Stream. The leptocephalus is planktonic and shaped like a willow tree leaf and drifts with the water movement of the current towards Europe a journey which may take three years (Tesch, 1977). The larvae spend little energy in swimming and appear to be planktonic. When the eel larvae reach the Continental Shelf, at the 1,000 m depth contour, another part of the great mystery takes place and the larvae metamorphosise reducing in length and becoming more rounded like adult eels (Schmidt, 1909). At this stage there is little or no pigment present and the eels are termed "glass eels". The glass eels are now capable of directional movement and can swim against the current and actively seek out fresh water. During the spring

months in Britain, huge shoals of elvers migrate up the rivers and over any land based obstacle to complete the life cycle.

1.4.5. EEL FARMING TECHNIQUES

Considering the complicated life cycle of the eel, the difficulties of completing the cycle in captivity can be appreciated. Eels in captivity have not been induced to spawn naturally. *Anguilla japonica* has been spawned by the injection of extract of salmon hypophysis in the female and by treating the male with synahorin (Yamamoto *et al.*, 1974). The eggs were fertilised in salt water at 23°C., hatched 38-45 hours later and the larvae lived for five days. Keeping the larvae alive under planktonic conditions for up to three years and feeding them on plankton has proved a very difficult system to develop. It is for this reason that the only method open to commercial eel farms is to catch elvers from the wild as they migrate up the rivers. Elver fishermen net the migrating elvers out of the rivers when the elvers are swimming upstream, usually at night using a torch to attract them. The elvers are then weaned onto food such as cod roe and chopped earth worms at the fish farm. Commercial pastes are also available. Once weaned, the fingerlings are transferred to the fattening side of the farm where they are grown on to market size on commercially available solid high protein diets for a period of up to two years. Eels are cannibalistic and grow at vastly different rates and it is essential that they are constantly graded (Seymour, 1984). The industry has evolved two

different producers; the elver producers who buy off the fishermen and grow the elvers to fingerlings (2-5g juvenile eels) and the ongrowers who fatten the fingerlings eels to a market size. This differentiation is practically worldwide.

1.5 OBJECTIVES OF THE STUDY

The major objective of this study was to produce a scientific understanding of factors effecting eel growth in closed recirculating aquaculture systems in order to maximize growth rates, minimize mortality and increase the economic efficiency and profitability of eel farming within the E.U.

Specific objectives for on-growing eels (10g+) were to:

- 1) Develop a system that recycled the water 100% with water added only to make good evaporative loss.
- 2) Develop techniques to assess short-term and long-term stress in eels under commercial aquaculture conditions.
- 3) Determine the effects of increased salinity and stocking density on growth rates and stress.
- 4) Develop practical methods to increase stocking densities and growth rates without increasing stress in order to increase the financial viability of eel farming.
- 5) Produce a scientific background for understanding and improving eel culture under closed recirculating conditions which, in commercial terms, can be translated into more effective and profitable eel farming.

CHAPTER 2

Water Quality and Treatment

CHAPTER 2

WATER QUALITY AND TREATMENT

2.1 INTRODUCTION

2.1.1. HISTORICAL BACKGROUND

Recycling water in aquaculture is evolving into an ever more complex science. The principle has its roots in the Victorian era when it became no longer acceptable to discharge raw sewage into rivers rendering them azoic. For example, the River Thames in London is referred to in Samuel Pepys' diary in the middle of the seventeenth century as having a "foul stench". Early attempts to treat the sewage led to the discovery of the principles involved in basic water treatment whereby organic waste is converted into simple inorganic compounds by heterotrophic bacteria, a process termed mineralisation. These inorganic compounds, especially those containing nitrogen, are further broken down by oxidizing bacteria into less toxic and freely available nutrients to plants. The same principles are applied in current aquaculture practice together with more advanced techniques adapted from the ornamental fish keeping sector. Aquarium techniques again have their roots in the Victorian era with the establishment of public aquaria:

1853 saw the doors open at London Zoo's aquaria, 1871 the Crystal Palace Aquaria and 1872 Brighton Aquarium which has survived to this day. Initially aeration was by steam power and water clarity

maintained by large quantities of live oysters. Since the end of the Second World War, as fish keeping became a recreational pursuit, the technology and knowledge has developed alongside the aquaculture industry. The commercial fish farming sector has thus benefited from many technologies developed by the hobbyist, for example, under gravel filters first used in ornamental aquaria formed the starting point for the commercial development of submerged biofilters.

The most important feature of any aquaculture system, and particularly recirculation systems, is to maintain the optimal conditions of water quality. The most important parameters of water quality will now be discussed together with their effects on fish should optimal conditions not be maintained.

2.1.2. WATER QUALITY

Water is sometimes referred to as the universal solvent and possesses a unique range of physical and chemical properties (dependant on the included compounds and discussed below) which may influence the ability of aquatic organisms, such as fish, to maintain homeostasis.

The changing of these properties can have an adverse effect on the health and performance of the fish even to the point of causing death. In natural ecosystems changes normally will be corrected and the water returned to a less toxic and more

acceptable state to the organisms that inhabit it. For example biological cycles, such as the nitrogen cycle, are responsible for detoxification, coupled to physical systems (e.g. aeration) and chemical systems (e.g. carbonate buffering). However if levels of these parameters increase significantly then the system may be unable to respond and breaks down with the organisms inhabiting the system malfunctioning and ultimately dying.

Ideally, to the commercial fish farmer, an oligotrophic environment is desirable where there are no inorganic substances (referred to as nutrients in this area of study). The microbial production of these nutrients lowers the carrying capacity of the water by adding to the B.O.D. and in high concentrations some nutrients may be toxic to the fish. Initially when a recirculation plant is set up it is free of nutrients . However after the addition of food which is processed by the fish into various waste products, the water undergoes a eutrophication process leaving a high concentration of nutrients. These nutrients must be removed thereby maintaining a suitable environment for the successful culture of the fish involved in the system.

A brief account of the more important water quality parameters is given below, followed by a description of the system designed to maintain the required water quality.

2.1.2.a Nitrogen

Perhaps the compounds causing most concern in recirculating systems are the nitrogen compounds. Atmospheric or molecular nitrogen although omnipresent is of little importance as it is an inert gas. It can be of some concern if introduced to aquaculture systems under pressure such as from a leak in a pump inlet drawing in air. Such supersaturated nitrogen gas can cause death by gas bubble disease as it comes out of solution in the blood a situation similar to decompression sickness sometimes referred to as the "bends" in subaqua divers.

Of much more concern to the commercial aquaculturist are the major detrimental effects of the nitrogen containing compounds such as ammonia, nitrite and nitrate as a result of protein metabolism and nitrification by nitrifying bacteria (see section 2.1.3.b).

Examples of other nitrogen containing compounds entering into the system are proteins, amino acids, urea (CH_4ON_2) and uric acid ($\text{C}_5\text{H}_4\text{O}_3\text{N}_4$). These compounds are invariably broken down into ammonia by the mineralisation process of heterotrophic bacteria and then enter the nitrification cycle to be removed from the system.

2.1.2.b Ammonia

Ammonia is the primary nitrogen compound excreted as a result of protein metabolism especially in teleost fish fed on high protein diets. Ammonia occurs in the natural aquatic environment in small

amounts as a result of excretions of fish. Increased concentrations may occur mainly from pollution especially from sewerage discharge or agricultural silage, manure or fertilizer leaking into the water course.

In fish, ammonia originates in the liver and is excreted through the gills via the blood system (Smith, 1929; Wood 1958; Goldstein *et al.*, 1964; Janicki & Lingus, 1970). Deamination of plasma amino acids in the gill tissue is another source (Smith, 1930; Goldstein & Forster, 1961; Goldstein *et al.*, 1964). Ammonia is transported across the gills by two mechanisms; by diffusion down the concentration gradient in the case of the unionised form (NH_3) and also by active transport by exchanging the ionised form (NH_4^+) for a similarly charged ion in the exterior environment such as sodium ions (Maetz & Garcia-Romeu, 1964; Whitelaw, 1973). Similar exchanges have been observed in euryhaline teleost fish adapted to sea water (Motaïs, 1970; Evans, 1973).

Ammonia exists in two forms: ionised NH_4^+ and unionised NH_3 . The two forms, the sum of which is termed the total ammonia. Concentrations referred to in terms of nitrogen ($\text{NH}_3\text{-N}$) can be converted to NH_3 by multiplying by 0.8235 (Meade, 1985) and $\text{NH}_4^+\text{-N}$ is converted to NH_4^+ by multiplying by 1.3 (Spotte, 1979). Total ammonia exists in an equilibrium which is dependent on pH, temperature and dissolved oxygen concentration (Downing and Merckens, 1955). This is described in equation 2:1.

Equation 2:1.



Lowering the pH will drive the equilibrium in equation 2:1 to the right, one pH unit increase causes the percentage of un-ionised ammonia to rise by approximately tenfold (Spotte, 1979). The 24h-LC₅₀ of total ammonia nitrogen for the eel *Anguilla japonica* at 25°C. was estimated by Yamagata and Niwa (1982) to be 2,844 mg/l at pH 5, 820 mg/l at pH 7 and 16.8 mg/l at pH 9. By keeping the pH below 7 (see table 2:1 for fresh water, similar results are displayed by Whitefield (1974) in sea water) the total ammonia can be, in effect, stored in a non-toxic form until removed from the system by the nitrifying bacteria in the bio-filter (see section 2.1.3c). Increasing the temperature will increase the relative concentration of NH₃ at constant pH (table 2:1) as will decreasing salinity although this is a less dramatic effect than increasing pH. It is suggested by Sousa *et al.* (1974) using chinook salmon smolts (*Oncorhynchus tshawytscha*) that not only is it practical to shift the ammonia into the ionised form by decreasing the pH but the toxicity can also be reduced by using intermediate salinities. Their reasoning is that this salinity dependent reduction in toxicity may be related to an ionic exchange relationship between sodium and ammonium across the cellular membranes of the gill branchial cells.

TABLE 2:1

Variation in % NH_3 in an Aqueous
Ammonia Solution With Temperature
and pH

After Emerson *et al.* (1975)

VARIATION IN % NH_3 IN AN AQUEOUS AMMONIA SOLUTION WITH
TEMPERATURE AND PH

Temp. (°C.)	pH Range							
	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5
0	0.008	0.026	0.083	0.261	0.820	2.55	20.7	45.3
5	0.013	0.040	0.125	0.400	1.23	3.80	28.3	55.6
10	0.019	0.059	0.186	0.590	1.83	5.56	37.1	65.1
15	0.027	0.087	0.273	0.860	2.67	7.97	46.4	73.3
20	0.040	0.125	0.400	1.24	3.82	11.2	55.7	79.9
25	0.057	0.180	0.570	1.77	5.38	15.3	64.3	85.1
30	0.081	0.254	0.800	2.48	7.46	20.3	71.8	89.0

The toxicity of ammonia is of much debate; it is however generally agreed that the unionised form is extremely toxic while the ionised form is considerably less toxic (Smart, 1981; Yamagata & Niwa, 1982). Sadler (1981) concludes that, in the European eel *Anguilla anguilla*, elvers are more susceptible to NH_3 than mature eels with growth being halted at a concentration of 0.5 mg/l $\text{NH}_3\text{-N}$. Some toxicity has been attributed to the ionised form NH_4^+ in the Japanese eel *Anguilla japonica* although this is in effect negligible (Yamagata & Niwa, 1982). Thurston *et al.* (1981) speculated that NH_3 is 300 to 400 times as toxic as NH_4^+ .

2.1.2.c Nitrite

Recently, the effects of nitrite in fish has been of increasing interest, possibly due to the trend in the aquaculture industry towards recirculation of water. Its effects are well studied in man and higher vertebrates leading to the World Health Organisation recommending maximum dietary intakes of less than 1 mg/l as N-NO_2 (WHO, 1978) ($\text{N-NO}_2 * 3.3 = \text{NO}_2$ (Spotte, 1979)).

Nitrite is present in recirculation systems as an oxidatory product of the breakdown of ammonia by the bacterium *Nitrosomonas sp.* In the natural aquatic ecosystem nitrite is present mainly as a product of the reduction of nitrate by the activity of phytoplankton and some atmospheric nitrogen is fixed by lightning and oxidized to both nitrite and nitrate. Normal levels in the environment vary but typically range from 1-5 mg/l. Levels of 5-20 mg/l have been recorded in a survey of 65 Italian lakes

previously considered to be unpolluted (IRSLA 1980). 10 mg/l is considered to be a warning sign of pollution (EIFAC 1984). Generally, poor quality salmonid fisheries are associated with high nitrite levels of 60-200 µg/l although this is not evident in coarse fisheries (Solbe, 1981).

Nitrite exists in an equilibrium according to equation 2:2

Equation 2:2



The reaction is pH dependant with the relative concentration of HNO_2^- , the conjugate acid, being decreased as the pH is decreased. As the temperature of the water rises, the disassociation constant of the HNO_2^- increases and hence the relative concentration of NO_2^- rises. Both temperature and pH effects are minimal in the system used in the present study as, for example, at pH 7 12.5°C., the relative concentration of NO_2^- is 99.978% and at the same temperature at pH 6, the NO_2^- relative concentration is 99.781 (EIFAC, 1984). Therefore at the temperature and pH values used in typical recirculating aquaculture systems (pH 7 and temperature 20-25°C) the relative concentration of NO_2 is practically 100%

One of the reasons that nitrite is well studied in man is due to a condition called methaemoglobinaemia which can be caused by the iron in haemoglobin, normally being in the divalent (Fe_2^+) form,

being oxidised by nitrite to the trivalent(Fe_3^+) form when it forms methaemoglobin. Methaemoglobin is unable to combine reversibly with oxygen in the blood as occurs with haemoglobin and the result, in severe cases, is anaemic hypoxia. In fish normally 5% of the haemoglobin is in the form of methaemoglobin, (Eddy *et al.*, 1983) and this is maintained constant by a methaemoglobin reductase in the red blood cells.

High levels of haemoglobin in fish appear to be of less importance than in higher vertebrates. In experimentally induced high concentrations of plasma methaemoglobin (by the increase of nitrite concentrations), over 70% methaemoglobin in rainbow trout *Oncorhynchus mykiss* (Smith and Williams, 1974) and chinook salmon *Oncorhynchus tshawytscha* (Brown and McLeay, 1975), did not induce mortalities. The fish were however stressed. Some Antarctic fish, having no haemoglobin at all, rely on the plasma to carry the oxygen as the reduced temperature allows a high plasma concentration of dissolved oxygen, (Holeton, 1970).

Early studies on the toxicity of nitrite in fish gave widely varying results until Perrone and Meade (1977) discovered that the chloride ion concentration in the environment strongly counteracted the toxicity of nitrite in coho salmon *Oncorhynchus kisutch*. This was confirmed later in rainbow trout *Oncorhynchus mykiss* (Bath and Eddy, 1980). The ratio of chloride to nitrite is critical and protection can be given against nitrite toxicity to the fish by increasing the chloride concentration in the environment. A $\text{Cl}^-/\text{N.NO}_2^-$ ratio of 15:1 in rainbow trout *O.*

mykiss is sufficient to render nitrite non toxic (Bath and Eddy, 1980). A figure of 18:1 has the same effect in coho salmon *Oncorhynchus kisutch* (Perrone and Meade, 1977) and 41 for the channel catfish *Ictalurus punctatus* (Tomasso et al., 1979).

Nitrite in the environment enters the fish through the gills via the branchial epithelial cells in rainbow trout *Oncorhynchus mykiss* (Gaino et al., 1984). The concentration of methaemoglobin in the blood of fish has been shown to closely follow the trend of nitrite in the aquatic environment with the gills concentrating the nitrite up to ten times the external level in the blood and tissues (Eddy et al., 1983).

Rainbow trout *Oncorhynchus mykiss* exposed to 1.5 mg/l nitrite (0.45 mg/l N-NO_2^-) for 72 hours showed inhibited lysosomal activity in the liver and fragile lysosomal membranes. Liver damage was observed which was typical of that caused by anaemic hypoxia induced by methaemoglobin (Mensi et al., 1982). Plasma concentrations of corticosteroids in channel catfish *Ictalurus punctatus* were raised after exposure to ammonia and nitrite (Tomasso et al., 1981). Fish recovered more rapidly when ammonia rather than nitrite was the cause of the plasma corticosteroid increase. A concentration of 5 mg/l NO_2^- (1.5 mg/l N-NO_2^-) in the environmental water was sufficient to raise the corticosteroid level by ten times the normal amount.

The toxicity of nitrite can be enhanced by its reaction with certain amines causing the formation of compounds such as

N-nitrosodimethylamine which when fed to rats at a level of 5 ppm caused tumours in 70% of the experimental group (Wolff and Wasserman, 1972). This effect is uninvestigated in fish.

Environmental pH is another important factor affecting NO₂- toxicity with an increase in pH reducing its toxicity (Wedermeger and Yasutake, 1978). Over a pH range of 6.4-9.1 the toxicity of nitrite has been found to decrease as the pH is increased (Russo *et al.*, 1981).

2.1.2.d Nitrate

Comparatively little is known about the effect of nitrate in intensive aquaculture although it has been speculated that pale gills in captive fish are a result of elevated nitrate in the environment (Spotte, 1979). Levels of 400 mg/l have been found to have no effect on the mortality and growth in the fresh water large mouth bass *Micropterus salmoides* and channel catfish *Ictalurus punctatus* (Knepp and Arkin, 1973). It is claimed that nitrate is 2,000 times less toxic than nitrite in coho salmon *Oncorhynchus kisutch* and rainbow trout *Oncorhynchus mykiss* kept in fresh water, with no sign of stress after exposure to 1,000 mg/l for over 5 days. Toxicity increased by up to 1.41 times in brackish water at 15 ppt. (Westin, 1974). Nitrate is considered harmless at relatively large concentrations in eels with 24 hour LC50 3,130 mg/l for the Japanese eel *Anguilla japonica* and no acute toxicity reported at concentrations below 1,150 mg/l (Mie *et al.*, 1984).

In the natural environment DOP is released from dead or ruptured animal cells or excreted by macroalgae. Heterotrophic bacteria mineralize DOP into DIP. DIP is precipitated out into calcium salts (Goldizen, 1970) or calcium and magnesium salts (Saeki, 1962). In sea water, in the presence of magnesium and ionic ammonia, the mineral struvite ($\text{NH}_4\text{MgPO}_4 \cdot 6\text{H}_2\text{O}$) is formed by certain bacteria (Malone and Towe, 1970). The DIP is removed by precipitation in an albeit minor way. In fresh water DIP combines with the ferrous ion to precipitate out (Adey and Loveland, 1991).

In an aquaculture system, fish faeces and uneaten food contain DIP and POP, POP is converted into DIP by heterotrophic bacteria. DIP can be physically removed by aeration; the phosphate bound to organic molecules is adsorbed into air bubbles and dissipated as aerosol droplets at the surface when the bubbles burst (Sutcliffe *et al.*, 1963).

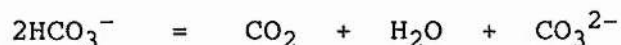
The phosphate is removed at a rate proportional to its concentration (Bayler *et al.* 1962). Thus:

$$C_t = C_0 e^{-kt}$$

where C_t is the concentration of reactive phosphate at time t ; C_0 is the concentration of reactive phosphate at the beginning time 0; e is the base of natural logarithms and k is a rate constant dependant on the surface area created by the bubbles.

system according to equation 2:3. Some CO₂ is therefore maintained within the system.

Equation 2:3



The equation is pH sensitive (Weyl, 1970) and offers very little buffering at normal sea water pH. The maximum buffering being at pH 6 and 9 (Skirrow, 1975).

In a natural environment, the CO₂ is reduced back into organic carbon by plants. Bicarbonate can also be photosynthetically reduced into organic carbon by algae (Blinks, 1963).

D.O.C. has been attributed to the yellow pigment in sea water and is termed "Gelbstoff" by Kalle (1966). Although Gelbstoff is largely of plant origin it would appear to have an animal content which together with nitrogenous wastes could account for the persistent yellow/brown colour characteristic in many recycling fish farm waters.

2.1.2.f Phosphorous

There are three forms of phosphorous: dissolved inorganic phosphorous (DIP and also termed phosphate, orthophosphate or reactive phosphate), dissolved organic phosphate (DOP) and particulate organic phosphate (POP).

In the natural environment DOP is released from dead or ruptured animal cells or excreted by macroalgae. Heterotrophic bacteria mineralize DOP into DIP. DIP is precipitated out into calcium salts (Goldizen, 1970) or calcium and magnesium salts (Saeki, 1962). In sea water, in the presence of magnesium and ionic ammonia, the mineral struvite ($\text{NH}_4\text{MgPO}_4 \cdot 6\text{H}_2\text{O}$) is formed by certain bacteria (Malone and Towe, 1970). The DIP is removed by precipitation in an albeit minor way. In fresh water DIP combines with the ferrous ion to precipitate out (Adey and Loveland, 1991).

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where C_t is the concentration of reactive phosphate at time t ; C_0 is the concentration of reactive phosphate at the beginning time 0; e is the base of natural logarithms and k is a rate constant dependant on the surface area created by the bubbles.

DIP levels in recycling systems stabilise very quickly, rarely exceeding 3 mg/l, and not much concern was given to removing it in the system used in this study as it was considered harmless.

2.1.2.g Oxygen

Oxygen makes up 21% of the atmosphere but is relatively insoluble in water the solubility decreasing with temperature rise, increased salinity, a reduction in barometric pressure (increased altitude) and impurities (see table 2:2).

The oxygen consumption in fish depends on many variables which include;

Temperature - fish consume more oxygen at higher temperatures due to increased metabolic activity

Dissolved oxygen (DO) in the water - increased DO increases the diffusional gradient across the gills.

Activity - the more active the fish the more oxygen consumed.

Feeding - more oxygen is consumed both during the activity of feeding and post feeding, in digestion and assimilation. For example oxygen demand doubles one to six hours after feeding in channel catfish (Tucker and Robinson, 1990).

Ammonia - high levels of ammonia cause large and prolonged increases in post feeding oxygen consumption. For example as reported in 1-2 g. eels *Anguilla anguilla* (Knights, 1989).

TABLE 2:2

Solubility of Oxygen in Fresh and Sea Water
at 100% Saturation

After Shepherd and Bromage (1989)

SOLUBILITY OF OXYGEN IN FRESH AND SEA WATER
AT 100% SATURATION

Temp (°C.)	Solubility in fresh water mg/l	Solubility in sea water(35 p.p.t.) mg/l
0	14.6	11.3
5	12.8	10.0
10	11.3	9.0
15	10.2	8.1
20	9.2	7.4
25	8.4	6.7
30	7.6	6.1
35	7.1	5.7
40	6.6	5.3

Size of fish - the smaller the fish the more oxygen per unit body weight the fish utilises. The oxygen consumption of eels with reference to size in a farm situation is given by Stahler GmbH (Hadamar, Germany) as follows:

Weight (g)	O ₂ Consumption (mg/kg/h)
1	1,100
10	600
50	420
300	250

Species - minimum required levels of oxygen are higher for salmonids (5.0-5.5 mg/l for fish and 7.0 mg/l for eggs) than for species such as carp, catfish and tilapia which can withstand DO levels below 2.0 mg/l for periods of about 12 hours (Shepherd and Bromage, 1992) and adult channel catfish can survive short periods at less than 0.5 mg/l (Tucker and Robinson, 1990).

Minimum oxygen levels vary for a particular species according to the variables listed above. Oxygen concentration in water may be expressed either as % saturation or as mg/l and is dependant on environmental water temperatures. Colt and Orwicz (1991) suggest 90% saturated water to be the minimum. Fish are reported to exhibit poor growth at DO concentrations of less than 25% (Romaine 1985). When 2-5g eels (*Anguilla japonica*) were exposed to DO levels of less than 40% saturation, growth feeding and food conversion rates decreased but were not affected when maintained

at 60% saturated water (Rowchai *et al.*, 1986). An oxygen concentration of about 1 mg/l at 25°C. produced high mortalities in 2g. eels *Anguilla japonica* (Yamagata *et al.*, 1983). As oxygen levels decrease, fish will respond by decreasing their activity, cessation of feeding and eventually "gasping" at the surface. Oxygen starved fish eventually die due to a decreased pH in the blood as a result of increased glycolysis to meet energy demands due to stress. Decreased pH disables the efficiency of haemoglobin to extract oxygen from the blood.

The bacteria in the biofilter are essentially aerobic. How much oxygen they require is the source of some debate. The sea water bacterium *Nitrocystis oceanus* is capable of nitrification at oxygen levels as low as 0.05 mg/l (Gundersen, 1966). Kawai *et al.* (1971) demonstrated that total ammonia concentration in fresh and sea water was maintained at an acceptable level (0.465 mg/l) at an oxygen concentration of 34% saturation whereas at DO levels of 6% saturation, the total ammonia was 173.6 mg/l.

2.1.2.h pH

pH is the negative logarithm of the hydrogen ion concentration. In mathematical terms the prefix p denotes a negative logarithm of the following term, pH is therefore $-\log_{10}[\text{H}^+]$. Pure water ionises only very slightly producing 10^{-7} moles of hydrogen and hydroxyl ions per litre. The pH of pure water at 25°C. is therefore 7. At pH values above 7 the hydroxyl ion predominates and the water is alkaline (the hydrogen ion concentration being less than 10^{-7}). At pH values below 7 the hydrogen ion

predominates and the water is acidic (hydrogen ion concentration greater than 10^{-7}). As the pH scale is logarithmic, each decrease of one unit represents a ten fold increase in hydrogen ion concentration.

Extremes of pH in natural fresh water range from 5-10 (Boyd, 1990) and sea water remains constant at a pH of approximately 8.2 due to the bicarbonate buffering system. Acid rain producing mineral acids, salts and weak bases plus other man made pollutants are recent phenomena in industrial environments which are responsible for decreasing pH values of natural water courses. Other factors affecting pH in natural waters are CO_2 from respiration of fish and other aquatic organisms, carbonic acids from soils, swamps forests and bogs and mineralisation and nitrification in organic decay in bottom sediments. The pH value of natural ecosystems increases during the day due to phytoplankton utilizing CO_2 during daylight to perform photosynthesis and decreases during darkness due to the phytoplankton excreting CO_2 during respiration. The effects of changes in pH on the environment are important in all biological systems including in recirculating aquaculture systems. Perhaps the most important effect in this respect is that of pH on the ionised/unionised condition of ammonia as outlined above. However the concentration of hydrogen ions has an effect on all chemical dissociation equilibria in the chemistry of water, the most important of which are covered in this chapter.

Effects of pH on fish have been observed in salmonids below pH 5 where the fish start to lose the ability to regulate plasma sodium concentration leading to the loss of integrated body movements due to drastically reduced sodium chloride concentrations in the plasma (Leivestad and Muniz, 1976).

In recirculation systems pH is constantly decreasing due to the heterotrophic microorganisms and the release of hydrogen ions by nitrifying bacteria as ammonia is reduced to nitrate. This process is more apparent in soft water as the buffering capacity is weak (Bisogni and Timmons, 1991.)

The effect of pH on the biofilter is equally important in a recirculation system. The preferred pH is slightly higher than neutral with minimum requirements of pH 6.5-7 (Petit, 1990) below this the bacteria can slowly adapt to a range of pH 5-10 but cannot respond to rapid changes of more than 0.5-1 pH unit (Wheaton *et al.*, 1991).

2.1.2.1 Alkalinity

Alkalinity is a measure of total concentration of titratable bases expressed as millequivalents per litre or meq/l. Alkalinity has been simplified by expressing it as mg/l of equivalent calcium carbonate (CaCO_3) thereby standardising the many bases (mostly CO_3^- and HCO_3^-), one meq/l equals approximately 50 mg/l CaCO_3 . The total alkalinity reflects the buffering capacity of the water by neutralising any addition of carbon dioxide and repressing any fluctuations in hydrogen ions thus maintaining a

stable pH. Acceptable limits for aquaculture are reported to be 20-400 mg/l CaCO_3 (Tucker and Robinson, 1990) any less leading to a depletion in CO_2 and a resultant rise in pH (Sawyer and McCarty, 1978). A minimum of 40 mg/l is recommended for the efficient functioning of biofilters (Paz, 1984).

2.1.2.j Hardness

Hardness is the total concentration of metal ions expressed in meq/l or in terms of mg/l of equivalent calcium carbonate (CaCO_3). The main metal ions are magnesium (Mg^{2+}) and calcium (Ca^{2+}) with lesser amounts of iron and manganese. Equal amounts of hardness and alkalinity are preferred to keep the pH stable (Romaine, 1985). The recommended levels of hardness are 20-300 mg/l (Boyd and Walley, 1975). Invertebrates are particularly affected by soft water where a certain amount of calcium availability is required for shell formation although this is not a problem in sea water with an average hardness of 6,600 mg/l (Boyd, 1990).

Calcium and sodium have been shown to have a sedative effect on fish in high concentrations and also in certain concentrations lead to a reduction in oxygen absorption and mucous secretion. Calcium is known for its reduction of the toxic effects of nitrite (Perrone and Meade, 1977; Bath and Eddy, 1980) similar to that described for chloride ions (see section 2.1.2c). Heavy metals are usually toxic and care should be exercised to avoid copper and brass fittings in recirculation systems.

2.1.2.k Temperature

Teleost fish inhabit waters from below 0°C. to 45°C. although individual species have a preferred temperature range where the enzymatic activity in the fish is at an optimum for maximum growth and performance. A temperature above the upper limit of thermal tolerance will result in death as the life supporting enzymes cannot function; similarly a temperature below the limit of thermal tolerance leads to reduction in enzyme activity. In particular sudden changes in temperature will kill fish due to "thermal shock". Certain species may have limited physiological control over body temperature through processes such as countercurrent heat exchange mechanisms between arteries and veins and the ability to reduce circulation to the surface of the body. In the wild, fish are capable of limited body temperature control by certain behavioural responses, such as migration. Under cultured conditions fish are totally reliant on the temperature controlled by the aquaculturist.

Although the optimum temperature from the farmer's point of view is that at which the fish grows most rapidly, and usually at the upper end of the range to which the fish is tolerant, it is important to note that diseases are more active at high temperatures. For example vibriosis, caused by the bacterium *Vibrio anguillarum*, is associated with increased environmental temperatures and does not appear to infect salmonids and turbot below 10-11°C. and Anguillidae and Pleuronectids below 15-16°C (Roberts, 1989). Conversely, low temperatures have certain diseases associated with them such as the disease caused by the

bacterial group known as myxobacteria producing thickening of fins, due to epithelial hyperplasia, and eventual necrosis; this condition is termed "cold water disease". Some other common temperature related problems are:

- 1) Wounded fish, although capable of healing faster at high temperatures, they are in greater danger of bacterial and fungal infections.
- 2) Increased temperatures can heighten the fish's susceptibility to toxins.
- 3) Oxygen, as mentioned in section 2.1.2.g, is less soluble at higher temperatures.
- 4) Fish, having a higher metabolic rate at high temperatures, have a higher oxygen demand and care must be exercised to ensure that it is met.

Temperature affects all the chemical reactions mentioned in this chapter which are essential in maintaining a balance in managing a recycling unit. The rate of a chemical reaction approximately doubles with a 10°C rise and so the recirculating system must be carefully observed as the temperature is increased.

2.1.3 BIOFILTRATION

Section 2.1.2 on water quality outlines the preferred levels of some of the more important constituents of the water in a recirculating system (see table 2:3 for a summary).

TABLE 2:3

Water Quality Parameters - Maximum
and Minimum Recommended Levels in
a Closed Recirculating Aquaculture
System

Water Quality Parameter	Minimum Level Recommended	Level Maintained In Study	Maximum Level Recomended
Ammonia mg/l	0	0-3	> 8
Nitrite mg/l	0	1-5	> 10
Nitrate mg/l	0	< 500	> 500
Oxygen mg/l	4	5-8	14
pH	6	6.5-7.5	8.5
Alkalinity mg/l CaCO_3	20	> 7	400
Hardness mg/l CaCO_3	20	> 9.3	300
Salinity ppt	0	12	35
Temperature $^{\circ}\text{C}.$	20	23	28

When water is first introduced to a recirculation system it is essentially free from solutes but as time advances after fish are introduced, the water will become increasingly hypernitrified. This system will eventually break down and become toxic resulting in death of all the fish, unless the water is treated to reduce this toxicity.

Biofiltration is the process whereby naturally occurring microorganisms are used to convert toxic compounds in the water into less toxic compounds which are ultimately removed from the system. In order to understand the functioning of the biofiltration process, the basic principles are outlined below.

2.1.3.a Mineralisation

Mineralisation is a general term used to refer to the removal of unwanted organic compounds from the system. Organic compounds increase in the system as a result of waste excretion from fish and other living organisms, uneaten food, lysis the cells of dead microorganisms. Mineralisation results from the activity of heterotrophic bacteria and algae in natural water systems although the action of the bacteria are reported to exceed that of the algae (Munro and Brock, 1968). During the process complex organic molecules are broken down into simple inorganic compounds; eg proteins into amino acids and ultimately to ammonia, and carbohydrates into carbon dioxide and water. Bacteria can use amino acids as a carbon source by transamination, dehydrogenation, decarboxylation and deamination resulting in organic acids, ammonia, carbon dioxide and various

amines. Depending on the species of bacteria, one or more of these methods may be employed.

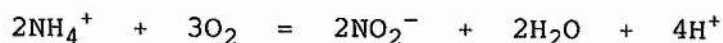
As the concentration of organic matter rises, so does the biomass of heterotrophic bacteria. There is, however, a finite limit to this process and the upper limit is reached when the respiratory requirements of the bacteria are not matched by the availability of oxygen in the culture system.

Heterotrophic bacteria do more than just break down organic compounds into inorganic ones and have been reported to be actually compete with nitrifying bacteria by using nitrogen as an energy source (Johnson and Sieburth, 1976). 104 species of soil heterotrophs have been observed to be capable of oxidising ammonia to nitrite (Crump, 1933) and some can oxidise nitrogenous organic compounds directly to nitrite and nitrate without producing the intermediate ammonia (Jensen and Gundersen, 1955).

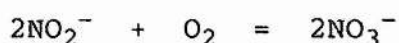
2.1.3.b Nitrification

Nitrification is one of the main processes in the biofilter. As discussed in section 2.1.2b, ammonia is excreted by the fish and has to be removed as it is highly toxic. This process is performed by bacteria of the genus *Nitrosomonas* which oxidise ammonia to nitrite (equation 2:4) which is in turn oxidised into nitrate by the bacterium *Nitrobacter* (equation 2:5).

Equation 2:4



Equation 2:5



Nitrifying bacteria are not obligate autotrophs but due to their ability to assimilate organic compounds are in fact facultative. Under certain conditions they can use significant amounts of carbon as a source of energy (Smith and Hoare, 1968).

Efficiency of nitrification can be impaired by several factors discussed separately below:

Toxins - many toxins affect nitrifying bacteria to various degrees but only those concerning recirculation systems are discussed here. The respiratory rate and growth of *Nitrosomonas* have been shown to be depressed by nitrite. *Nitrobacter* was shown to be sensitive to ionised ammonia and even more so to unionised ammonia and both types of bacteria were only slightly sensitive to nitrate (Meiklejohn, 1954).

Sulphide - sulphides are produced in anaerobic areas of the system and at concentrations of 0.03 mg/l have been shown to severely inhibit *Nitrosomonas* (Srna and Baggaley, 1975). At higher concentrations of 0.1 mg/l sulphide inhibits the conversion of nitrite to nitrate.

Effects of disease treatments - in conventional through flow fish farming methods, many chemicals have been used to combat various diseases and a wide range have been developed to combat fish

diseases. However the addition of these compounds to recirculation systems can, to varying degrees, have an adverse affect on the biofilter's bacterial population. The effects of some of the commonly used anti disease treatments on biofilter capacity has been studied by Collins *et al.* (1975; 1976) and Levine and Meade (1976); table 2:4 summarises their findings.

Oxygen - According to the results presented by Kawai *et al.* (1971) it would be unwise to allow the water in the biofilter to drop below 34% of saturation. The oxygen concentrations in this study are reported in section 2.3.5.

pH - upper and lower limits of pH are discussed in the section on water quality. According to Petit (1990) the optimum pH is 6.5-7 but nitrifying bacteria will adapt to ranges of pH 5-10 (Wheaton *et al.*, 1991).

Temperature - optimum range for *Nitrosomonas sp.* is 10-40°C. (Kawai *et al.*, 1965) inhibition occurs at less than 5°C. (Buswell *et al.*, 1954). Optimum range for *Nitrobacter sp.* is 4-45°C. and inhibition at less than 4°C. (Painter, 1970).

TABLE 2:4

Commonly Used Compounds Used in
the Treatment of Fish Diseases
and Their Effect on the Nitrification
Capacity of the Biofilter

DISEASE TREATMENT COMPOUNDS COMMONLY USED IN FISH CULTURE AND
THEIR INHIBITARY EFFECTS ON NITRIFICATION

Compound	Conc. (mg/l)	% Inhibition of Nitrification	Source
Chloramphenicol	50	84	Levine and Meade(1976)
	50	0	Collins <i>et al.</i> (1976)
Oxytetracycline	50	0	Collins <i>et al.</i> (1976)
Sulfamerazine	50	0	Collins <i>et al.</i> (1976)
Sulphadiazine	25	74	Levine and Meade(1976)
Sulphanilamide	25	65	Levine and Meade(1976)
Erythromycin	50	100	Collins <i>et al.</i> (1976)
Nifurpirinol	1	0	Collins <i>et al.</i> (1976)
	0.1	20	Levine and Meade(1976)
	4	44	Levine and Meade(1976)
Chlorotetracycline	10	76	Levine and Meade(1976)
Formalin	10	0	Collins <i>et al.</i> (1975)
	15	27	Levine and Meade(1976)
Furnace	0.1	20	Levine and Meade(1976)
Malachite green	0.1	0	Collins <i>et al.</i> (1975)
Mal.Gn. + Formalin	0.1 + 25	0	Collins <i>et al.</i> (1975)
Methylene blue	5	100	Collins <i>et al.</i> (1975)
	1	92	Levine and Meade(1976)
Copper sulphate	1	0	Collins <i>et al.</i> (1975)
	5	0	Levine and Meade(1976)
Potassium permanganate	4	0	Collins <i>et al.</i> (1976)
	1	86	Levine and Meade(1976)
Methanol	25-75	0-43	Levine and Meade(1976)
Sodium chloride	5 g/l	2	Levine and Meade(1976)

2.1.3.c Biofilters

Central to all water recycling systems is the biofilter. The chemical processes utilised by the respective types of bacteria have been described above and their effects can be harnessed in two basic ways; fixed film process and activated sludge. The biofilter is a vessel used to contain the various bacteria required to purify the water.

(i) Fixed film - These types of biofilter are by far the most common and are usually thought of as providing physical substrates for the development of autotrophic and heterotrophic bacteria. In reality they are a living habitat for many microorganisms including; cyanobacteria, ciliates, protozoans, fungi, bacteria and nematodes. Microorganisms that attach themselves to solid surfaces, or substrates, and are used in biofiltration are termed periphytes (ZoBell, 1970). Bacteria live in colonies and produce a slime in which other organisms such as nematodes can freely exist. The overall result of the complex community living on the biofiltrational substrate is to break down complex waste products.

The major function of the biofilter is to provide a substrate on which the microbial community can develop. The earliest forms of biofilter consisted of water percolating through gravel. The gravel provided a solid surface to which the bacteria could attach and a relatively large the surface area in comparison to the volume. Early filters were "wet-type" where the gravel was submerged. However these filters were easily blocked due to the

dead bacteria sloughing off, and could as a result become necrotic leading to the production of toxic compounds. A later development was the trickling filter whereby a high surface area to volume substrate (some being specifically manufactured) was held in a container and water was introduced from the top by a spray bar. Tricking filters were "dry" i.e. not submerged and introduced oxygen to the filter as the water trickled down the filter media. Fluidised bed filters were developed in which a substrate such as sand was kept fluid by passing the water to be treated, under pressure, from the bottom of the filtration tank to the top. Bio-rotators, of which the Stahlermatic filter used in this study is a type, were developed in an effort to overcome filter blockage due to necrosed bacteria. The filters have a series of plates which act as the substrate and rotate about an axis through the water to be treated.

(ii) Activated sludge - this is a process mainly used in the sewage treatment industry and is not as compact a method as fixed film reactors and is best suited to low volume high nutrient loading situations. The biofilter used in this study developed by Stahler GMBH, Germany differs from all other bio-rotators by incorporating this process in the design and this is described in section 2.2.1.a. The principle of the activated sludge process is aeration of the aqueous medium with a high organic load.

2.2 MATERIALS AND METHODS

The emphasis of this study was on commercial fish farming and as such the equipment used was based on scaled down production units. Careful consideration was given to the selection of the equipment and the design of the system which had to maintain water quality parameters within pre-defined limits.

2.2.1 PRINCIPLES OF OPERATION

2.2.1.a Biofilter

The Stahlermatic system used in this study combines the two water purification techniques discussed above (section 2.1.3.c). These systems are:

- (i) Activated sludge
- (ii) Fixed film processes on a submerged contact aerator.

The main component of the system is the Stahlermatic Biostage which consists of:

- (i) Stahlermatic Biotank
- (ii) Sedimentation tank which separates the purified water (supernatant), from the sludge which is re-cycled to the Biotank.

The Stahlermatic Biotank consists of a circular submerged contact aerator consisting of a series of Biodiscs (figures 2:1, 2:2

and 2:3). The aerator rotates around a central shaft powered by a motor installed above the water line and consists of profiled polypropylene or polyvinyl chloride plates (Biodiscs). These plates provide a large surface area to support the active biological fixed film. Rotation of the plates combines the two major processes of the water purification: activated sludge and fixed film processes. In comparison with conventional systems the Stahlermatic system exhibits three differences in design features:

1) High efficiency treatment of activated sludge:

An activated sludge is formed in the settlement tanks built onto either side of the biofilter (figure 2:1). This sludge has a high content of suspended solids consisting of waste products and uneaten food and forms a flocculant biomass containing autotrophic and heterotrophic bacterial colonies which detoxify the water.

Activated sludge is recycled directly from the sedimentation tank to the Biotank thus allowing growth of a distinct sludge which is continually treated.

FIGURE 2:1

Cross Section and Plan View of
the Stahlermatic Biofilter and
Clarifier

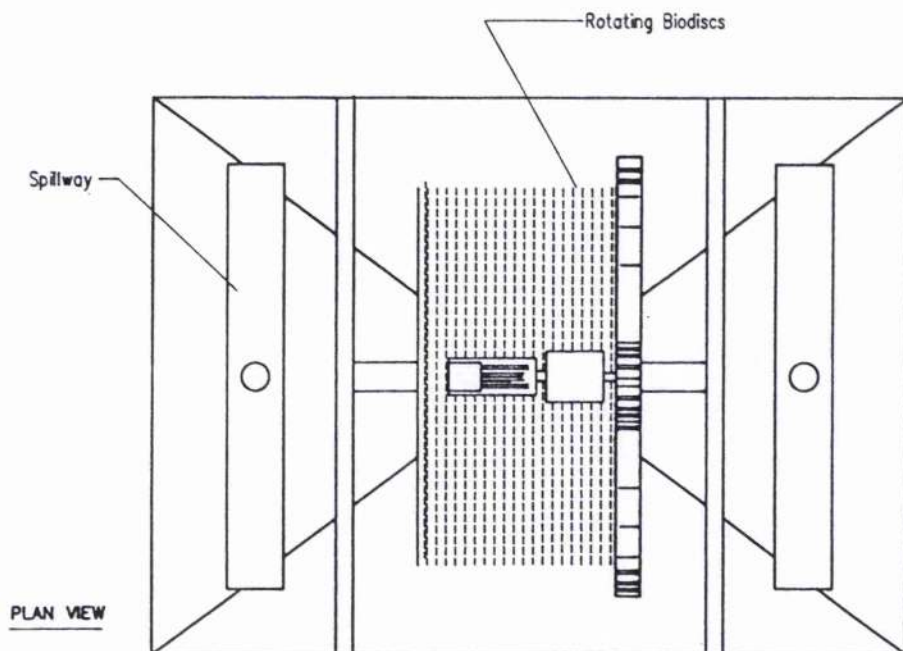
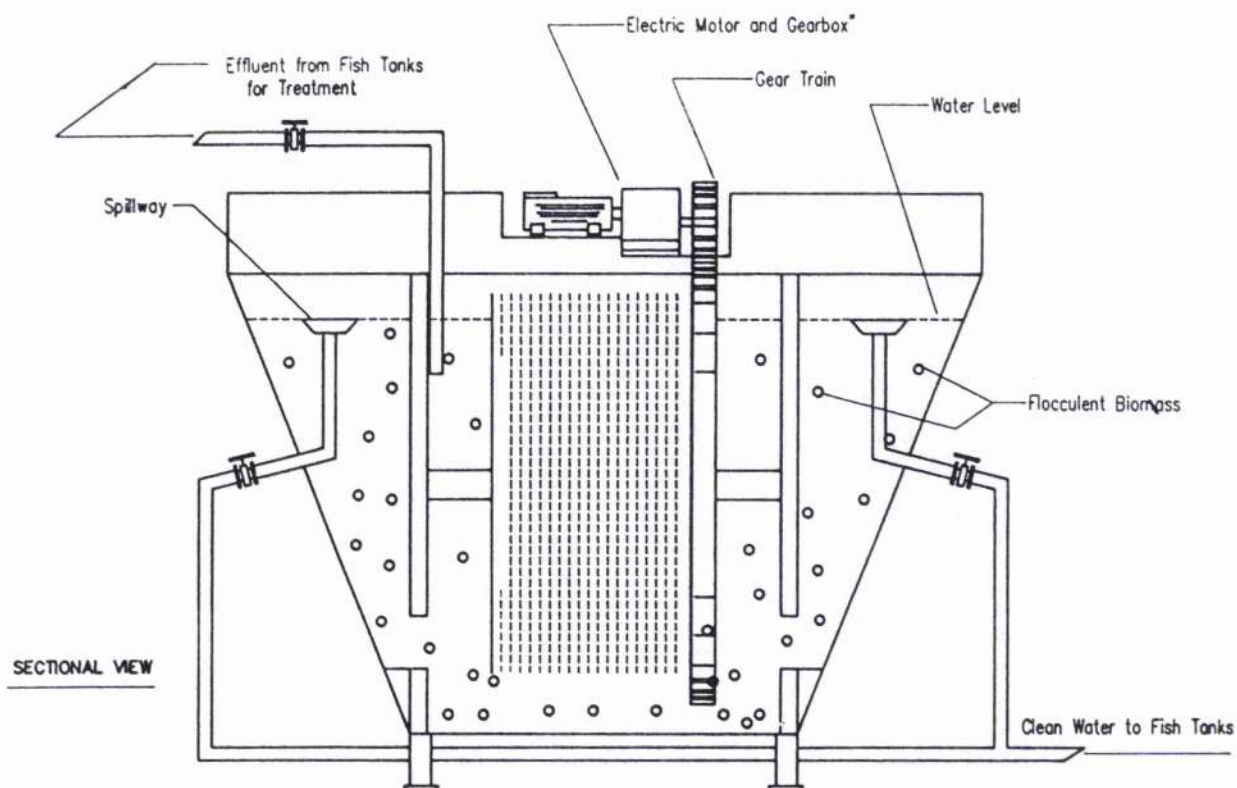


FIGURE 2:2

Individual Components of the
Stahlermatic Contact Aerator
of the Biofilter

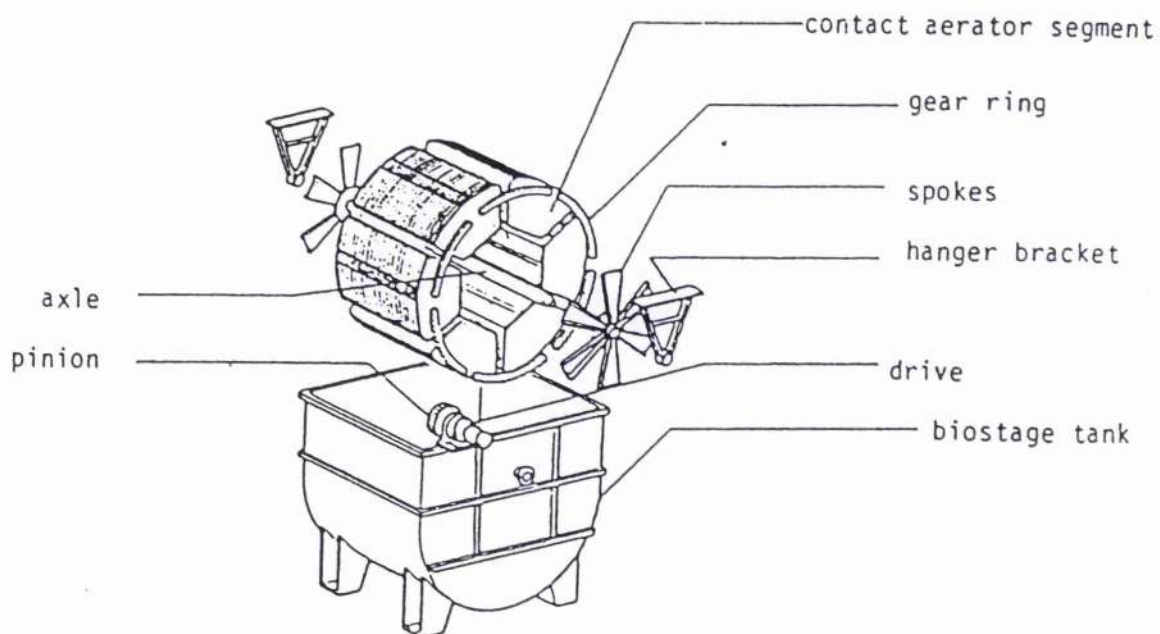
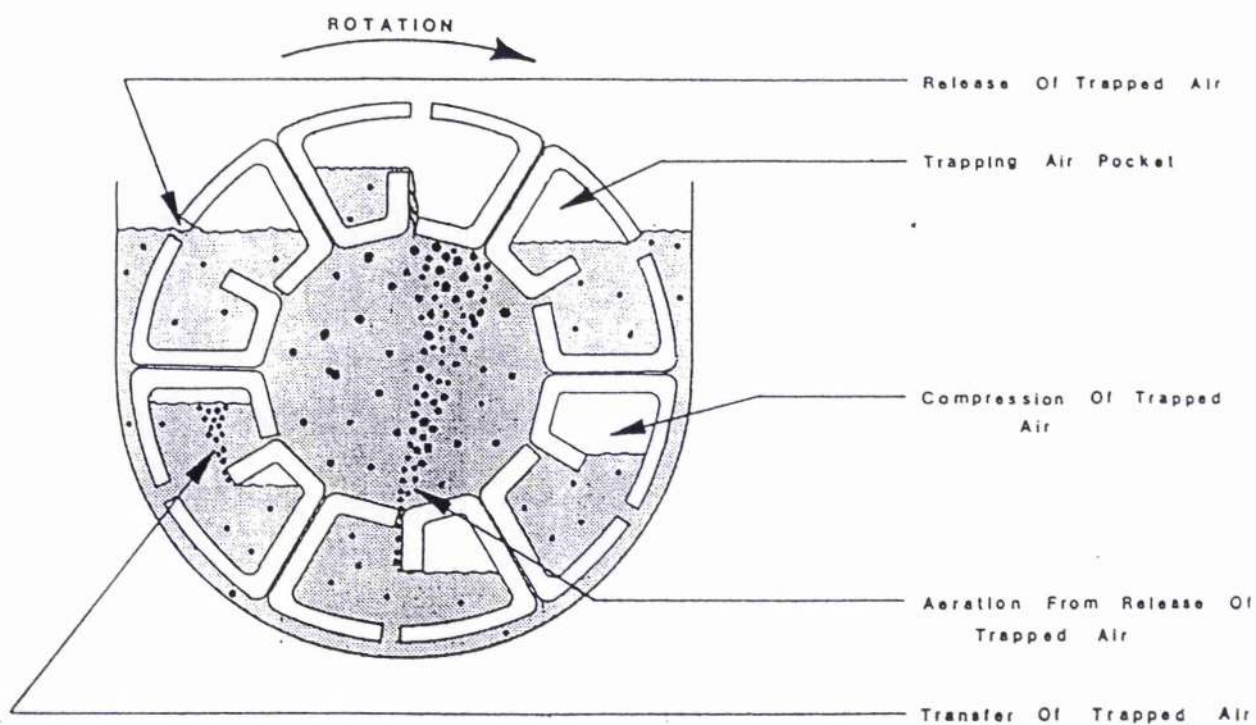


FIGURE 2:3

Cross Section of the Stahlermatic
Contact Aerator (Wheel) Displaying
Principles of Operation



2) Large biovolume :

The submerged biodisc is situated at a relatively deep level in the biotank thus effectively increasing the biovolume approximately two-fold compared to conventional biorotator systems. Twice the volume of water can be treated (see figure 2:3).

3) Effective oxygen supply:

The efficiency and capacity of a biological water treatment process depends upon a sufficient supply of oxygen. In conventional biorotator systems, oxygen is supplied to the fixed film as components pass through the atmosphere. Upon submersion, an oxygen gradient occurs from the fixed film to the water and oxygen is lost from the fixed film by diffusion. This reduces the effectiveness of the fixed film process and in addition the amount of oxygen supplied to the water is insufficient to support the growth of activated sludge. In the Stahlermatic system these problems are overcome by:

a) direct aeration of the water in the Biotank to an optimal level for growth of activated sludge. This also benefits the fixed film process as the oxygen content of the liquor in the tank is higher thus reducing diffusional loss of oxygen from the film.

b) the unique design of the Biodiscs which ensures optimal aeration of water by compression of air trapped in each segment (figure 2:3). Oxygen dissolves on the large surface area of the Biodisc and, due to the large concentration gradient, oxygen saturation occurs on the fixed film. As the wheel descends air

becomes trapped and compressed. In the fixed film process all parts of the profiled plates are in contact with air to ensure maximal oxygenation and in addition the activated sludge is oxygenated as the air becomes more compressed and escapes in the form of coarse and fine bubbles towards the centre of the wheel. The profile of each plate is designed to create the maximum number of bubbles thus increasing the number of interfaces at which exchange of oxygen can occur so increasing the oxidation of the liquor in the tank. Together with the turbulence created and rotation of the wheel, homogeneous mixing occurs thus maximising the activated sludge process.

2.2.1.b Oxygenation

Technical oxygen was added in small quantities to an oxibox (figure 2:4) which operated on the principle of introducing pure oxygen to water cascading over a series of discs in an enclosed vessel. The aeration system plus unique self aerating biodisc systems alone could have sustained the system but the oxygen was left in line as an extra safety feature.

2.2.1.c Foam fractionation unit

The structure of the foam fractionator (protein skimmer) is shown in figure 2:5. Dissolved or colloidal material is adsorbed onto the surface of a rising bubble as is commonly observed as foam on the sea in natural ecosystems.

FIGURE 2:4

Cross Section of "Oxibox" Aeration
Unit Displaying Principles of Operation

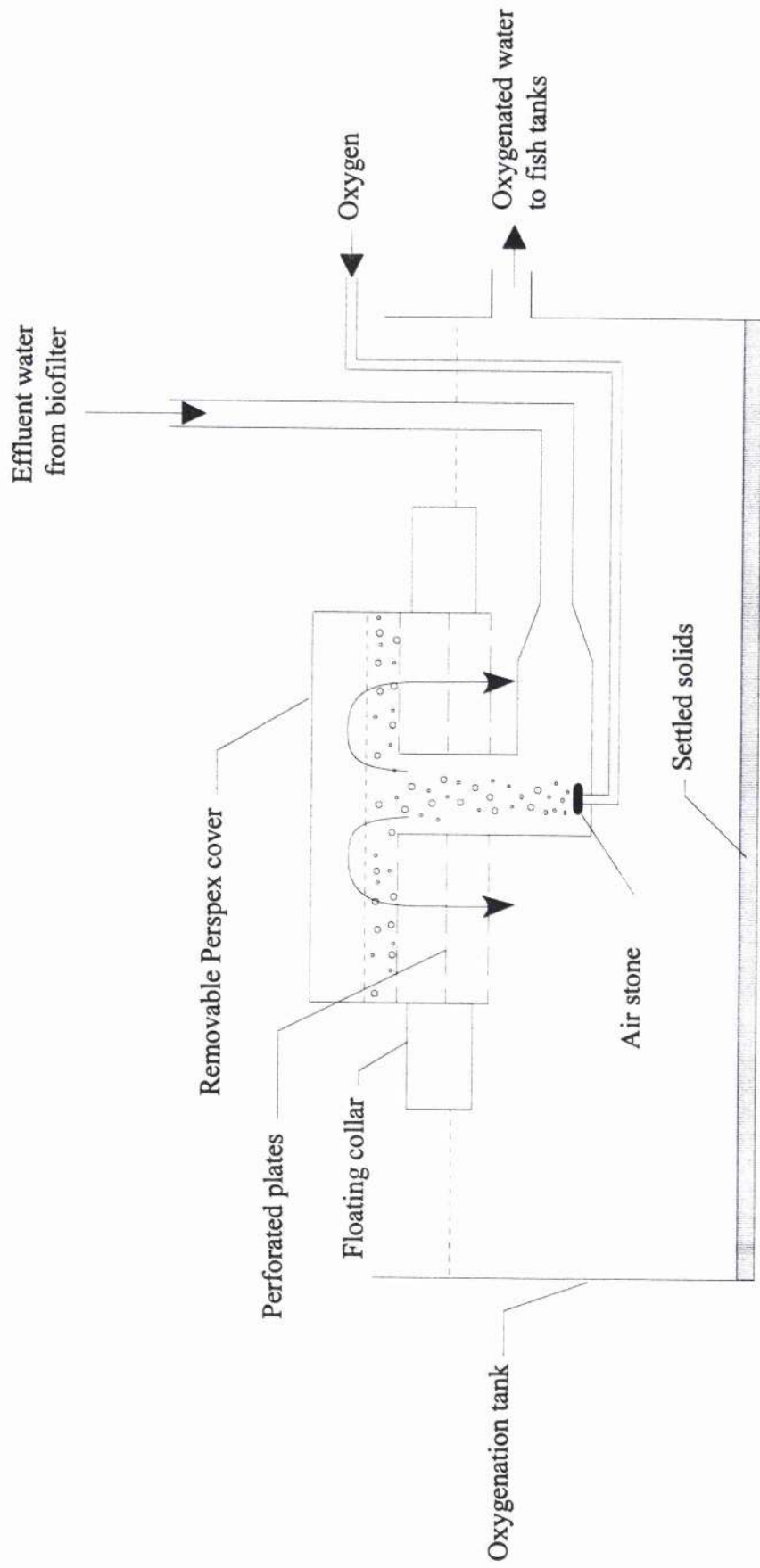
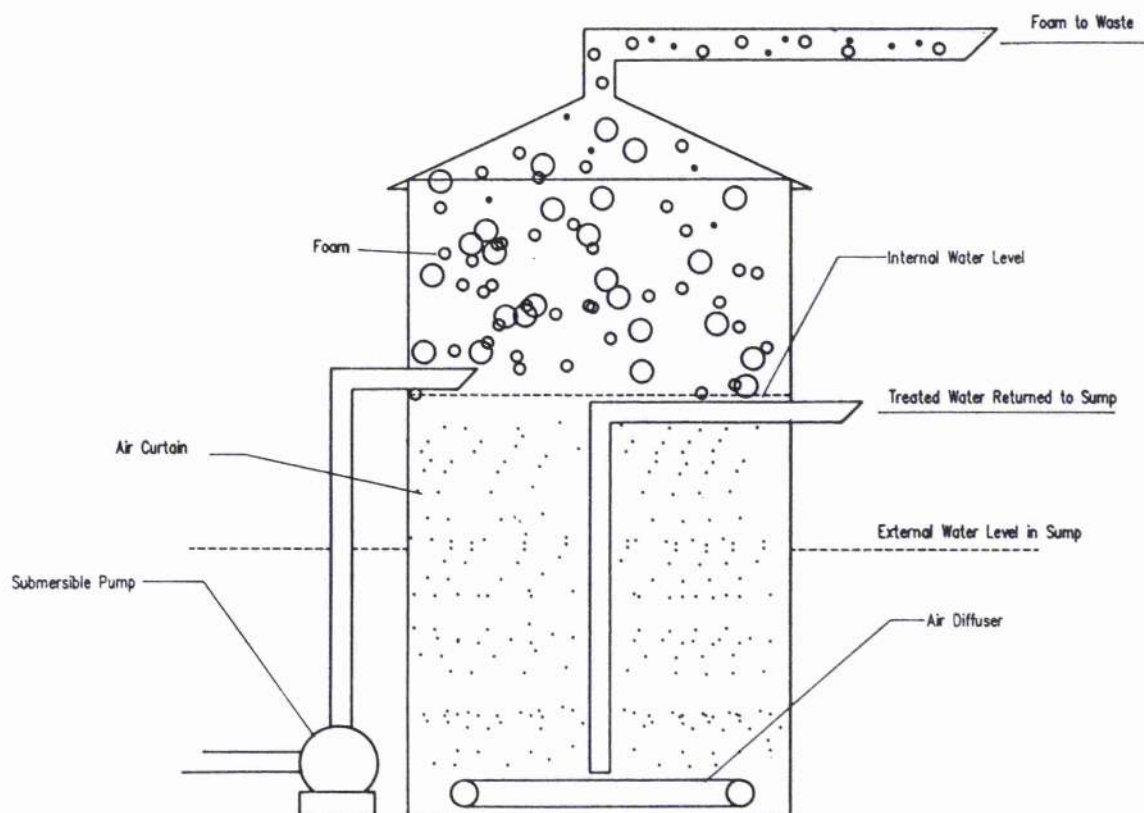


FIGURE 2:5

Cross Section of Foam Fractionation
Unit (Protein Skimmer) Displaying
Principles of Operation



Such complex molecules have a hydrophobic and hydrophilic region, the hydrophobic region attaches itself readily to the air/water interface and is removed as the bubble rises, reaches the surface and forms a foam; the subject is discussed in some detail by Lemlich (1972).

2.2.1.d Treatment of pathogens

One of the major advantages of recirculating aquaculture systems is that parasites and bacterial infection are not introduced from the flow of water experienced in conventional through-flow or cage farms. Throughout the experimental period very few problems arose but in exceptional cases the following treatments were used and all systems were treated in exactly the same way in order to maintain constant experimental conditions:

- a) Telmin paste - active ingredient Meybendazol at 200-300 mg/m³ for treatment of gill flukes.
- b) Formalin - (30% formalin solution) 138 ml/l maximum concentration used for controlling parasites and ciliate blooms.
- c) Malachite green - for treatment of fungal infection *Saproleginia* and *Ichtheophthirius*.

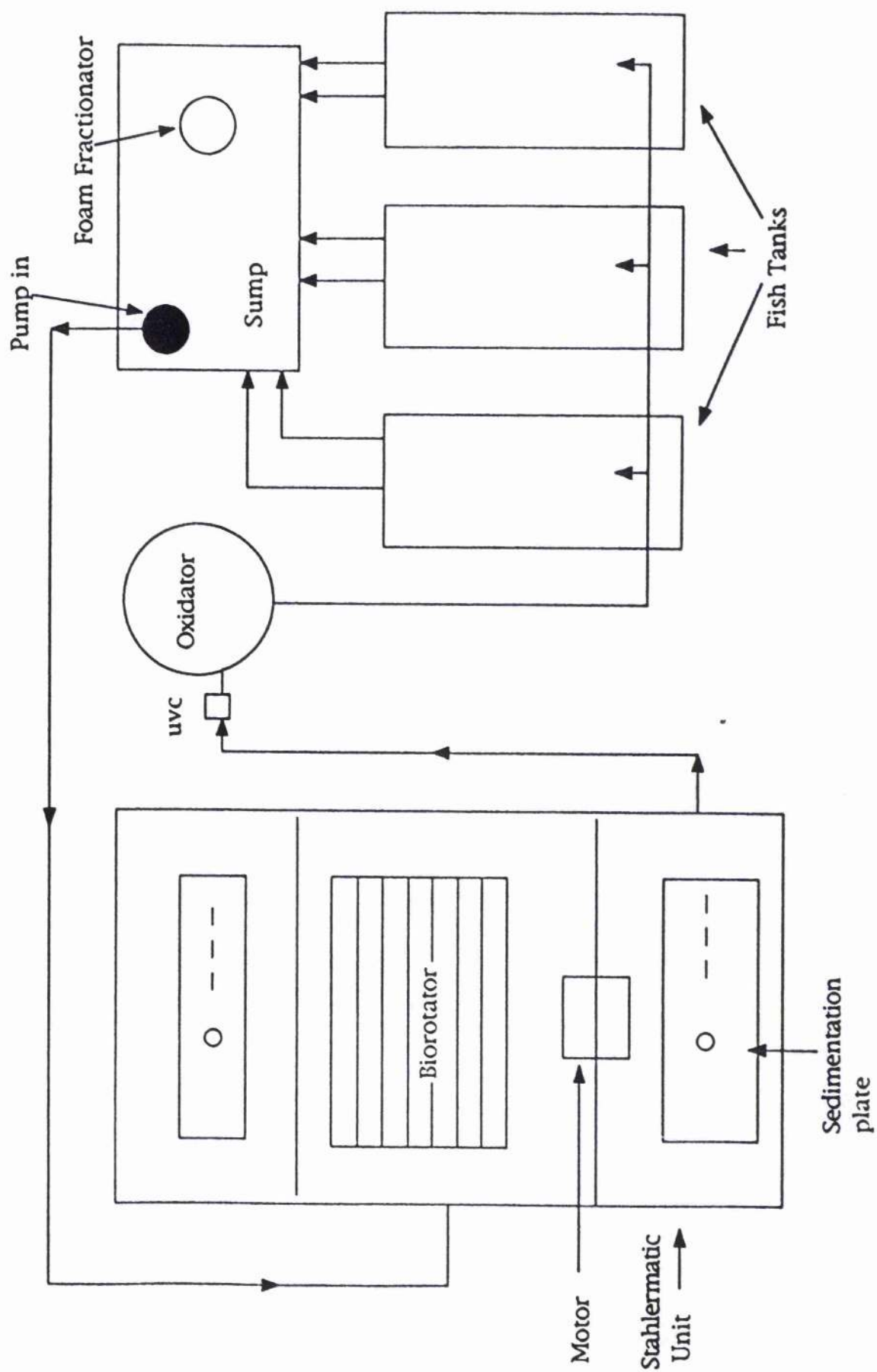
2.2.2 DESCRIPTION OF EQUIPMENT USED

2.2.2.a Experimental design

Two recirculating aquaculture systems were installed in an insulated industrial unit of total area 100 m². Figure 2:6 shows the layout of the plant. Each system consisted of a biostage (Stahlermatic Type 3) (figure 2:1) with a biotank (3 m³ capacity) and 2 clarifiers situated on either side of the biotank and connected to it (total volume 1.23 m³). The biotank contained 50 biodiscs which provided a total surface area of approximately 170 m² and rotated at a rate of approximately 2 revolutions/minute driven by an electric motor (300 W) geared down and the RPM controlled by an SEW Eurodrive variable frequency inverter Series 1000. The clarifier consisted of a spillway in each biotank which separated recycled water (supernatant) from sediment which subsequently flocculated and formed the activated sludge. Water exiting the biostage then passed into the oxidator which had a tank of volume 0.51 m³ containing an enclosed cascade-plate-type oxygenator (SanWa Oxibox) which was supplied with technical grade oxygen from bottles (BOC). Oxygenation was further maintained in the three fish tanks which each contained a "leaky-pipe" ring of 400 mm diameter located by plastic coated stainless steel cruciforms. The leaky pipe was supplied with compressed air from a ring main fed from a Compton two cylinder diaphragm oil free compressor.

FIGURE 2:6

Schematic Diagram of the Closed
Recirculating Aquaculture System
Used in The Study



Having passed through the fish tanks, water was collected in a sump of 0.49 m³ volume and then pumped back to the biostages via a submersible pump (Lowara DOC3 300 W 25-125 l/min but valved down to 20 l/min). At high stocking densities it was necessary to have a foam fractionator to remove proteins in the water, this was free standing in the sump with its own pump to input water and return was by gravity (figure 2:5). The temperature of the water was maintained by a 3 Kw tungsten heater (Zentec Ltd) controlled by an A Tech Aquastat and slave relay (Zentec Ltd).

Each system was alarmed to detect any changes in optimal conditions:

1) High water level in the pump sump indicated failure of the pump and was detected by a float operated reed switch 339-730 (RS Components Ltd) located in the sump.

2) An air pressure operated switch 317-134 (RS Components Ltd.) was placed in the air supply line to detect compressor failure sensitive to a pressure less than 20 psi.

3) A mains operated relay (RS Components Ltd.) was connected to the electricity supply to detect power failure.

All these systems were connected to a Response SA117 autodialler (RS Components Ltd.) which, when activated, dialled a radio-pager (British Telecom) which was carried at all times. In

addition high and low temperature audible alarms were fitted as an integral part of the A Tech Aquastat temperature control system.

2.2.2.b Water analysis

Water quality parameters were monitored daily (except where indicated) using the following equipment:

- Ammonia - Tetra Test Ammonia (Ulrich Baensch GmbH).
- Nitrite - Quantofix Nitrite (Machery-Nagel).
- Nitrate - Quantofix Nitrate (Machery-Nagel).
- Oxygen - Solexpress DO₂ 9060 (Solex International).
- pH - pH Boy (Camlab).
- Alkalinity - Initial water alkalinity values were obtained from the local water and sewerage authority (Ross and Cromarty Water and Sewerage) at the start of the experiments and were not measured during the course of the experiment.
- Hardness - Initial water hardness values were obtained from the local water and sewerage authority (Ross and Cromarty Water and Sewerage) at the start of the experiments and were not measured during the course of the experiment.
- Salinity - Refraction salinometer (Bio-Marine Inc.).
- Temperature - A Tech Aquastat (Zentec Ltd.) providing a continuous digital read-out.

2.3. RESULTS

2.3.1. PARAMETERS MEASURED DURING THE DEVELOPMENT OF SYSTEMS

2.3.1.a Nitrogen Compounds

The filters in both systems were allowed to develop over an initial 35 day period (figures. 2:7 and 2:8 show the development as the filters were stabilising). The initial increase in NH_4 was followed rapidly by conversion to nitrite and subsequently to nitrates. After this initial development phase both systems stabilised and both freshwater and saline water systems were similar as described below.

2.3.1.b pH

At the start up of the system, after the introduction of the eels, the pH was approximately 7 and rose to a maximum of 7.9 with an increase in ammonia lagging slightly behind. As discussed in section 2.1.2.b, ammonia at high pH values exists as NH_3 which is toxic to fish. It was decided that should the pH rise to 8, hydrochloric acid (HCl) would be used to reduce the pH, however, this did not occur as the pH began to fall in line with reduced ammonia concentrations (see figures 2:9 and 2:10). This was presumably due to the release of H^+ as the ammonia was oxidised to nitrite by the developing colonies of *Nitrosomonas* bacteria.

FIGURE 2:7

Concentration of Ammonia (mg/l),
Nitrite (mg/l) and Nitrate (mg/l)
During the Development of the
Biofilter in Fresh Water

- ⊙ = Ammonia Concentration
- = Nitrite Concentration
- = Nitrate Concentration

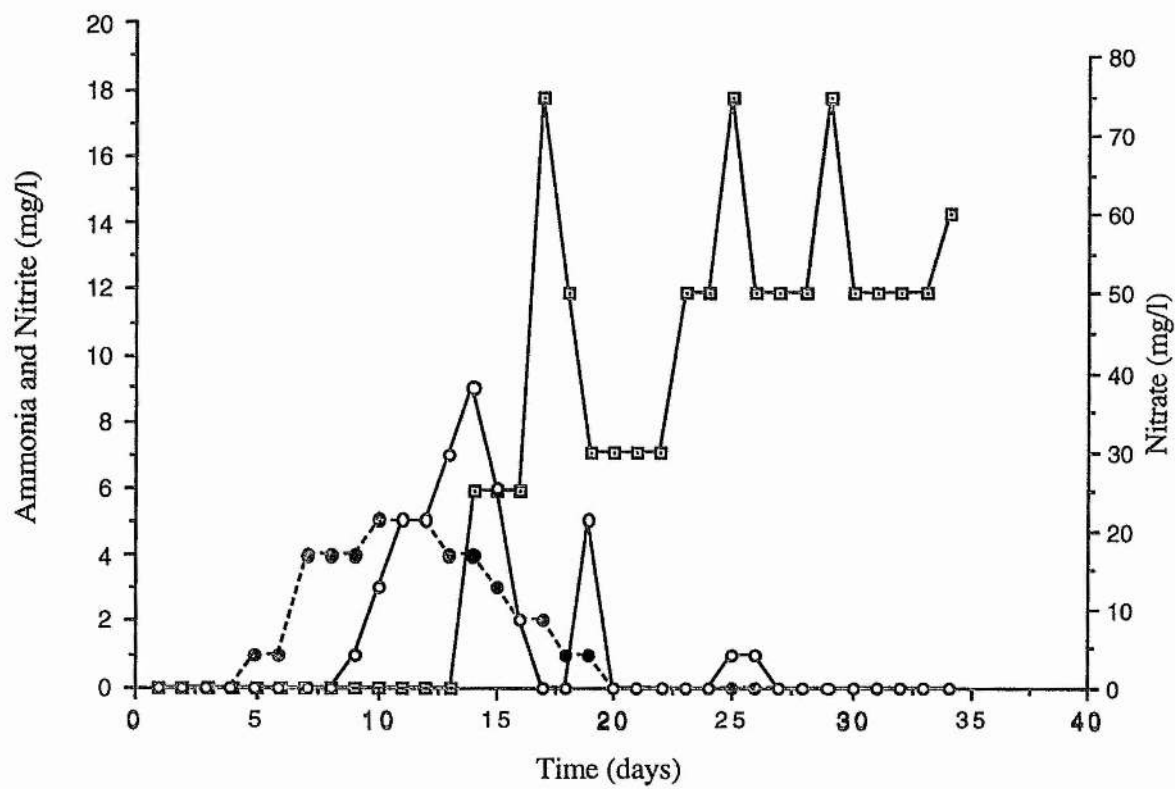


FIGURE 2:8

Concentration of Ammonia (mg/l),
Nitrite (mg/l) and Nitrate (mg/l)
During the Development of the
Biofilter in Saline Water (12 ppt

- ⊙ = Ammonia Concentration
- = Nitrite Concentration
- = Nitrate Concentration

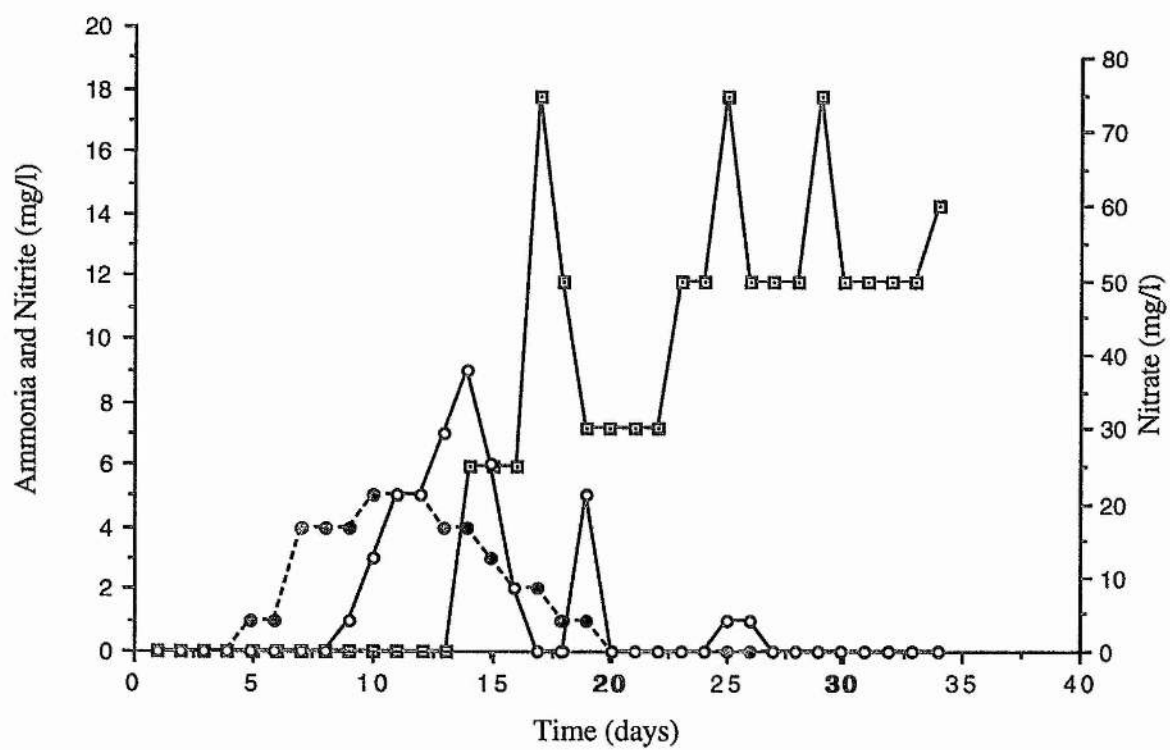


FIGURE 2:9

Concentration of Ammonia (mg/l)
and pH Values During the Development
of the Biolfilter in Fresh Water

o = Ammonia Concentration

⊙ = pH values

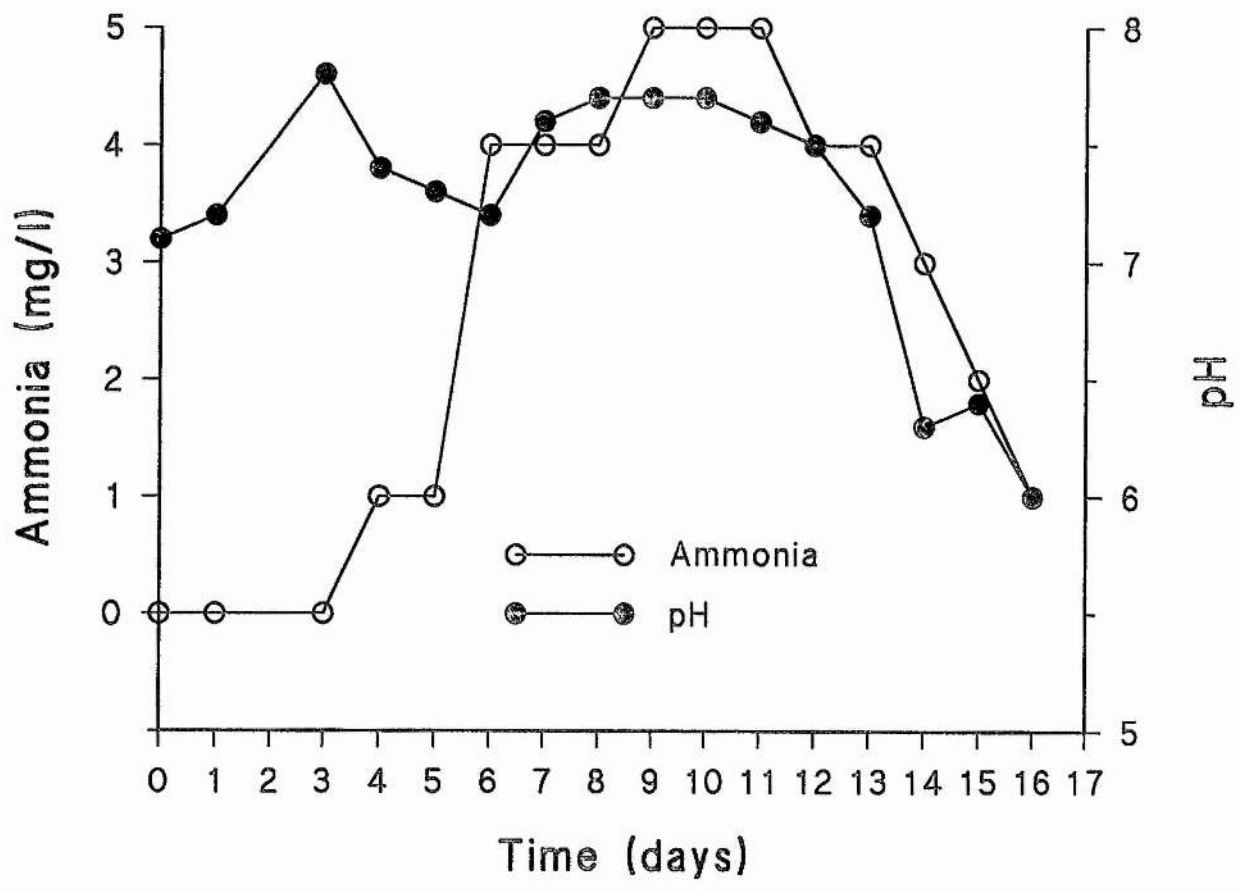
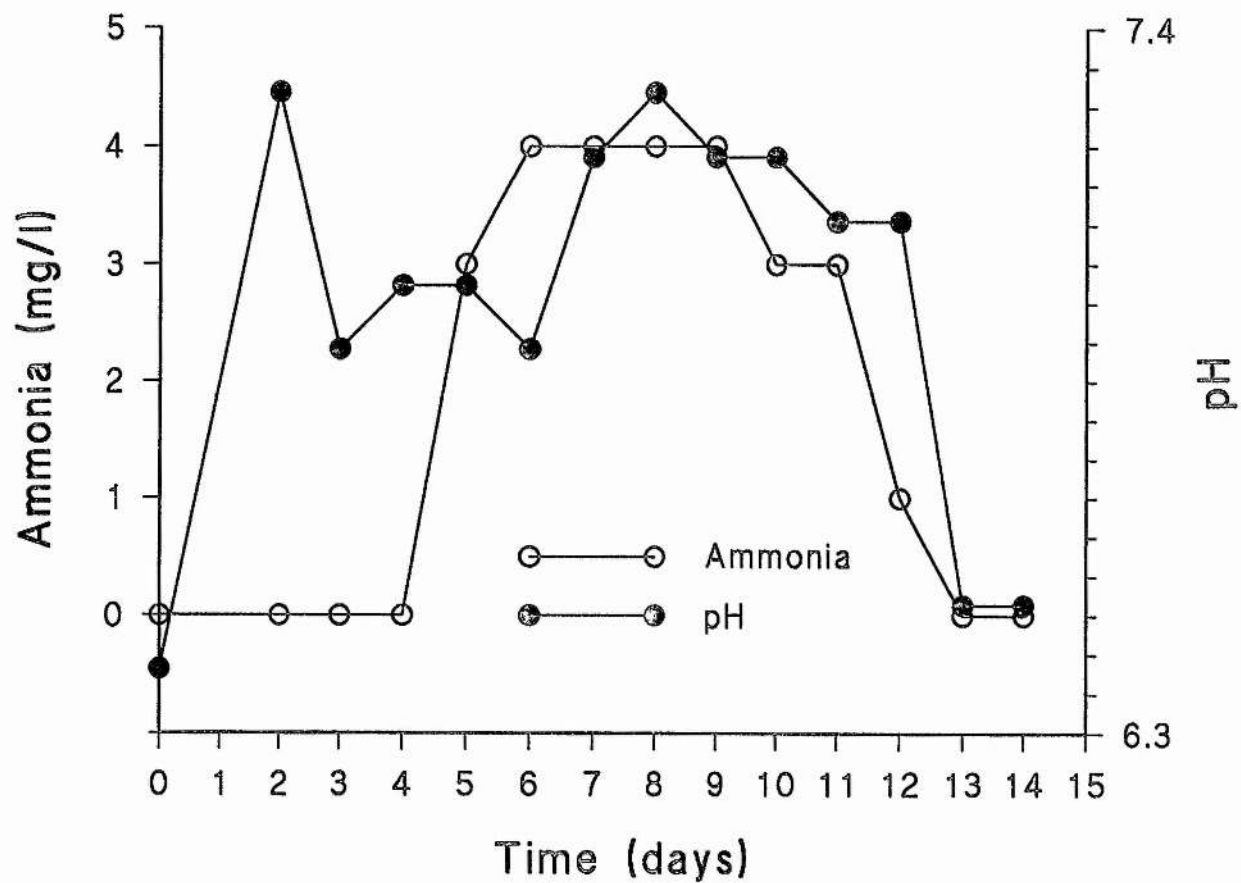


FIGURE 2:10

Concentration of Ammonia (mg/l)
and pH Values During the Development
of the Biofilter in Salt Water

o = Ammonia Concentration

• = pH values



2.3.1.c Alkalinity

The alkalinity of the water in the system commenced at 7.0 mg/l CaCO_3 and the water was therefore poorly buffered. No attempt to add buffering capability to the system was made as the pH was routinely monitored and adjusted. Alkalinity is considered to have little effect on fish themselves and was not measured during the course of the study.

2.3.1.d Hardness

Water hardness commenced at 9.3 mg/l CaCO_3 and would have, theoretically, increased with the addition of various compounds used to balance the pH and also by the addition of salt in the saline system. pH was adjusted by adding sodium hydrogen carbonate in preference to calcium hydroxide as the addition of calcium has been shown to increase the hardness over a period of time (Breder and Smith, 1932). Hardness was not measured during the course of this study as its effect on the fish and biofilter was considered to be of little concern.

2.3.2 PARAMETERS MEASURED IN FULLY DEVELOPED SYSTEMS

2.3.2.a Ammonia

In both the fresh water and saline water systems, the total ammonia concentration was typically less than 1 mg/l occasionally rising to 2 mg/l. Formalin (138 ml/l, 30%) was used in this study to control population explosions of ciliates. The biofilter gave a characteristic "kick" in response to these

treatments when the total ammonia concentration would rise from 0-2 mg/l to 3-5 mg/l over three to four days before returning to normal.

2.3.2.b Nitrite

Nitrite levels throughout the study were 1-5 mg/l in both fresh and saline water systems. An increase from this level to 4-7 mg/l was observed after the addition of formalin as described above. This increase in concentration did not occur until a few days after the formalin was added, presumably due to an increase in ammonia being oxidised by the *Nitrosomonas* sp.

2.3.2.c Nitrate

Nitrate levels initially rose steadily during the study before equilibrating at about 500 mg/l after approximately 85 days. In the fresh water system the nitrite rose initially faster. In the saline water system nitrate levels actually decreased to a concentration of approximately 150 mg/l towards the end of the growth rate experiment (approximately 200 days) possibly demonstrating the development of an additional bacterial colony with a capacity to breakdown nitrates. Anaerobic areas were encouraged within the system in an attempt to stimulate the growth bacteria which chemically reduce the nitrate ion by using it as an electron acceptor in the absence of the more powerful electron acceptor, oxygen e.g. *Micrococcus denitrificans*, *Pseudomonas denitrificans* and various other *Pseudomonas* sp. (see section 2.1.2d).

2.3.2.d Oxygen

Oxygen was maintained at a level of 14-19 mg/l (at 23°C.) at the inlet to all the experimental tanks throughout the duration of this study. Fish tank outlet DO levels varied between 5-8 mg/l. Oxygen levels in the biofilter were constant at around 100% saturation due to the unique self aerating effect of the Stahlermatic wheel (detailed in section 2.2.1.a). As a result it was not possible to assess the oxygen consumption of the biofilter by measuring influent and effluent oxygen concentrations. Oxygen consumption was impossible to determine in the fish tanks as direct aeration was supplied to the tanks and therefore inlet and outlet differences were meaningless.

On a single occasion when normal oxygenation was interrupted by a pipe connector failure, oxygen levels below 1 mg/l resulted in mortalities of 29.39% in the small population (17.18 g average weight). Levels of 3 mg/l Oxygen (as occurred during the occasional power cut, before the generator was put into the electrical system) produced signs of stress i.e. gasping at the surface but no mortalities.

The self aeration action of the biodiscs and the compressor operated aeration system were sufficient to meet the oxygen demands of the fish and the biofilter. Technical oxygen was supplied continuously via the "oxibox" (figure 2:4) as a safety measure and one BOC industrial sized bottle was consumed every 2-4 weeks.

2.3.2.e pH

pH was monitored daily and adjusted to keep it as near to pH 7 as possible. pH throughout the study decreased consistently and whenever the pH value fell to approximately 6.7, 135 g of sodium hydrogen carbonate (NaHCO_3) was added which increased the pH value to approximately 7.2. This process was found to be necessary approximately every two weeks. The water used was very soft with little inherent buffering capacity (7.0 mg CaCO_3 /l). Care was needed to ensure that the pH was not adjusted too rapidly as a sudden rise in pH could lead to a rapid rise in the unionised and highly toxic form of ammonia. For this reason the pH was never increased when the total ammonia level was greater than 4 mg/l. pH was maintained at 6.8–7.2 with exceptional absolute extremes of 6.5 and 7.8.

2.3.2.f Salinity

Salinity was monitored daily and remained constant in the salt water system at approximately 12 ppt. By continuously correcting for evaporative water loss the salinity was maintained at this constant level.

2.3.2.g Water Temperature

When determining the optimum temperature of the system, the requirements of both the fish and biofiltration biomass were considered. Optimum temperature for appetite and growth in eels *Anguilla anguilla* is reported as 26–27°C. (Seymour 1985), and this is considered to be the optimum functional temperature for

the digestive enzymes. For reasons of financial economy, temperature was thermostatically maintained at 23°C.

The second factor to be considered in recirculation systems with respect to temperature is the biomass of microorganisms, essential for detoxifying the water and removing unwanted excretory products. The temperature range at which *Nitrosomonas* (which convert ammonia to nitrite) are efficient is 10-40°C. with an optimum of 30-35°C. (Kawai *et al.*, 1965). *Nitrosomonas* are inhibited at less than 5°C. (Buswell *et al.*, 1954). *Nitrobacter* (which convert nitrite to nitrate) have an effective range of 4-45°C., optimum of 34-35°C. and are inhibited at less than 4°C. and greater than 45°C (Painter, 1970). Wortman and Wheaton (1991) found a linear relationship between temperature and both ammonia removal and nitrate production. A constant temperature of 23°C. was considered suitable for the bacteria in the biofilter.

Although temperature was maintained at 23°C., on one isolated occasion it was allowed to drop, during the 130 kg/m³ stocking density experiment, when a contaminated batch of salt was inadvertently added to the salt water system and all the water (7.25 m³) had to be changed. The heater was turned off for several days to allow the temperature to drop gradually to the same as the water used to replace it. The replacement water (with the uncontaminated salt added) was held in an unused system of the same volume and heated as the water in the other system cooled. The temperatures were equal (as one was going up

and the other down) at 17°C. All the water was replaced and the behaviour of the fish was apparently unaffected. For consistency the fresh water system was treated in the same way and all the water was replaced with fresh water.

2.3.2.h Water Quality

If the ammonia concentrations increased or water clarity decreased, it was discovered that restricting the feeding regime of the fish for a one or two day period was sufficient for ammonia levels to reduce to control levels (0-2 mg/l) or clarity to be restored. Both fresh water and saline water systems were unfed at the same time. The water quality was such that, apart from the water change due to a contaminated batch of salt (see section 2.3.2g), no water was required to be changed throughout the course of this study.

Water quality parameters maintained in this study and recommended maximum and minimum values, derived from the relevant literature for the culture of freshwater eels, are summarised in table 2:3.

2.4. DISCUSSION

The biofilters in both fresh and saline water were similar in their development and are represented in figures 2:7 and 2:8. Levels of ammonia rose first as the fish excretions built up within the system 3 days after the introduction of the fish, peaking after approximately 10 days. As the colonies of nitrifying bacteria developed in response to the ammonia substrate, nitrite was produced after 4-5 days peaking at approximately 13 days. Both ammonia and nitrite fell to stable levels of 1-2 mg/l (ammonia) and 1-5 mg/l (nitrite) and remained at these levels throughout the study unless the biofilter was disturbed by the addition of formalin (see sections 2.3.2a and 2.3.2b). Levels of nitrate (the end product of the nitrification process) rose more rapidly at first in the freshwater at a steady rate. This pattern of ammonia, nitrite and nitrate concentrations during the development of a biofilter is similar to that reported by Lawson (1995). Both systems reached a maximum nitrate concentration of approximately 500 mg/l after approximately 85 days.

The development of the biofilters is similar to the results obtained by Knosch (1991) using the Stahlermatic biofilter in a commercial eel farm. Data from a 100 tonne per annum farm using a trickling filter had higher ammonia (1-6 mg/l) and nitrite (1-10 mg/l) concentrations with much less stability (Knosche, 1991). The establishment of a stable nitrifying filter

requires 30-100 days (Lawson, 1995). Start up times can be reduced by 81% (4 days instead of 21 days) for ammonia and 89% (4 days compared with 37 days) by the addition of 10% wet media from an established biofilter (Bower and Turner, 1981).

The fall of the nitrate in the saline water system to 150 mg/l after 200 days (possibly due to the establishment of a colony of bacteria capable of breaking down nitrates) was interesting as nitrite removal is difficult under aquaculture conditions (see section 6.3.2h). Further detailed investigation would be needed to fully understand this result.

This study has demonstrated that careful design and knowledge of the biology of the system are essential for successful fish culture. Rather than being an exact science, as equipment manufacturers selling water purification systems have suggested, the operator must be able to predict changes in the biological demand of the fish and the fluctuating biological capacity of the biofilter. The operator is responsible for two biological systems; a) the fish and b) the microbial biomass in the water treatment side of the system, and must be sensitive to the requirements of both systems when attending to either one. For instance, increased ammonia cannot be immediately treated by the biomass in the biofilter as the bacteria have to divide in response to this rise and therefore increase in number in order to meet the demand. This situation will occur if the ammonia is the limiting nutrient. If however the oxygen is limiting, the bacterial biomass will not increase unless more oxygen is

supplied. When the ratio of $[O_2]/[NH_4-N]$ is below 3.6, oxygen is limiting whereas if it is above 3.6, the total ammonia is rate limiting (Heinsbroek and Kamstra, 1990).

It was discovered that increases in metabolites would require increased levels of treatment but only after a time lag as the necessary bacterial biomass increases. The subsequent decrease in metabolites will then become limiting and the biomass in the biofilter will reduce and the system then returned to the pre-crisis levels. The biological systems were therefore found, over a period of time, to have some degree of self regulation.

Response times to crises thus had to be learned. A deficiency in oxygen, due to compressor failure or increased demand, can be rapid and the time for the operator to respond and avoid mortalities is short (approximately one hour). However, once emergency measures have been implemented recovery is almost immediate (within 15 minutes). An increase in a metabolite such as ammonia may be more gradual as is the response of the biofilter which requires growth of the bacterial biomass (2-3 days). However temporary measures can be taken such as lowering the pH by the addition of hydrochloric acid (which causes the ammonia equilibrium to move to the non toxic ionised form as depicted in equation 2:1, section 2.1.2b), this effect is almost immediate. Similarly pump failure needs to be tended to within 4-5 hours to maintain circulation to the biofilter. Heater failure is not so serious and could be unattended to indefinitely with no danger to the survival of the fish. pH will

decrease gradually throughout the operation of the biofilter due to the release of H^+ during the oxidation process of nitrification (see section 2.1.3b, equation 2:4). This drop in pH does not present an immediate problem but is a chronic problem that needs to be monitored and adjusted accordingly (see section 2.3.2d).

Fish health problems vary in their urgency, with infestations of gill fluke *Gyrodactylus* at a low level being commonplace on an eel farm and of no great urgency in treatment. Scepticaemic conditions such as vibriosis can occur (although this did not happen in this study) and immediate treatment would be required with carefully chosen antibiotics so as not to affect the functioning of the biofilter (see table 2:4). Ciliate population explosions were regularly observed in fresh water and their treatment with formalin, although not urgent, must be timed with low concentrations of ammonia and nitrite as the treatment by formalin has a negative effect on the biofilter and may reduce the nitrification capacity for several days (see section 2.3.2a and 2.3.2b). The addition of formalin should also be timed with a period of low pH as the consequent reduction of biofiltration capacity leads to a rise in ammonia. This additional ammonia must be maintained in the relatively safe NH_4^+ form present at low pH values (see equation 2:1, section 2.1.2b). Other forms of treatment can have similar detrimental effects on biofilter performance (see table 2:4) and care in their use is required as in the use of formalin.

Recirculating systems require significant amounts of equipment and consequently the operator needs some engineering and electrical knowledge in order to run the system efficiently. However as this section has demonstrated, with careful monitoring and analysis of water conditions it is possible to operate 100% recirculation aquaculture under relatively constant conditions which allow for fish to grow rapidly. The optimal conditions for fish growth will be discussed in chapter 3.

CHAPTER 3

Growth Rates

CHAPTER 3

GROWTH RATES

3.1 OSMOTIC AND IONIC REGULATION

3.1.1 THE OSMOTIC AND IONIC ENVIRONMENT OF FISH

One of the aims of this study was to utilise the capacity of recirculation aquaculture systems to control environmental conditions. Salinity was varied to establish optimum growth conditions. In order to achieve this, it would be helpful to discuss the physiological mechanisms by which fish osmoregulate in different environmental salinities.

The salt concentration in the blood and body fluids of fish in both fresh and sea water is equal to approximately one third that of sea water (Eddy, 1981). Sea water is fairly constant worldwide at 34-36 ppt and the internal salt content of the fish is approximately 11-12 ppt. The internal salt content is regulated within narrow upper and lower limits in a healthy unstressed fish. Fish inhabiting either fresh or sea water environments are therefore presented with two different osmoregulatory problems. To the fresh water fish the environment in which it lives is hypoosmotic and it gains water osmotically and loses salts by diffusion. The converse is true in sea water fish which lose water and gain salt. Teleost fish combat these water and salt influxes and effluxes utilising the gills, pharynx, gut, urinary

bladder and kidney. The skin is relatively impermeable both osmotically and ionically (Fromm, 1968), supplied with mucus glands and protected by scales. Mucous itself is not impermeable but may reduce salt and water exchange to some extent. Chloride cells (section 3.1.3) have been observed in the skin of certain larval teleosts e.g. sardine *Sardinops caerulea* (Lasker and Threadgold, 1968) and ayu *Plecoglossus altivelis* (Hwang, 1990).

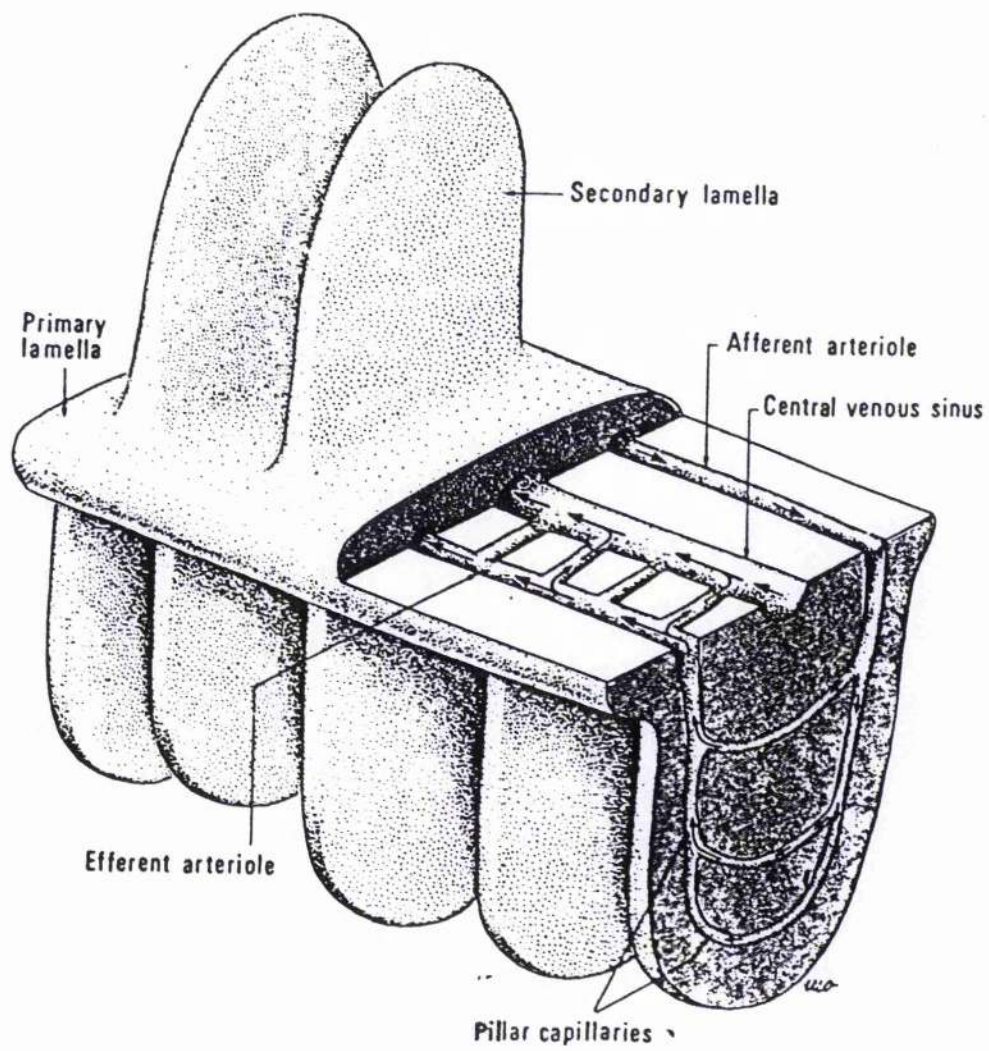
3.1.2 STRUCTURE AND FUNCTION OF THE TELEOST GILL

The gill plays a major role in osmoregulation in teleost fish. The basic structure consists of four branchial arches either side of the pharynx with a double row of primary lamellae at right angles supporting secondary lamellae on each of the two surfaces. Two types of epithelium are found in the gill; primary epithelium covering the primary lamellae and secondary epithelium covering the secondary lamellae, figure 3:1 (Laurent and Dunel, 1980). The primary epithelial layer is multilayered and is comprised of both respiratory cells and the mitochondrial rich chloride cells. The secondary epithelium is composed mainly of an outer mucosal layer of flat respiratory (pavement) cells 3-5 μm thick containing relatively few mitochondria and a prominent Golgi apparatus and rough endoplasmic reticulum (Maetz, 1971). Chloride cells (see below) have been observed in the secondary epithelium and may proliferate in response to a specific ion deficiency (Avella *et al.*, 1987). The inner epithelial layer is composed of a serosal layer of non differentiated cells.

FIGURE 3:1

Schematic Representation of Gill
Lamellae

(After Pisam *et al.*, 1987)



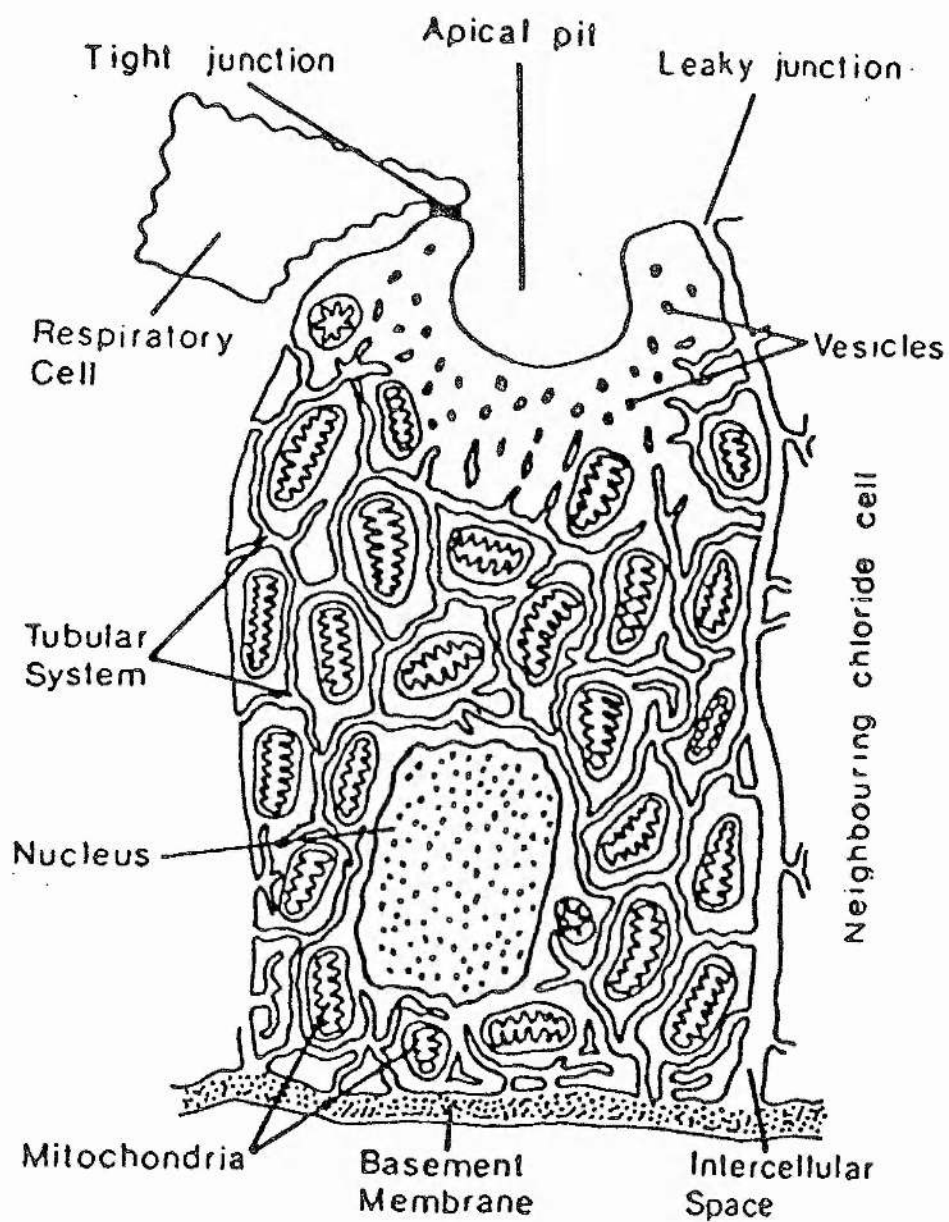
3.1.3 CHLORIDE CELLS

Chloride cells are the structures responsible for Na^+ and Cl^- exclusion and are found in larger quantities in marine teleosts than in fresh water teleost fish in the gill primary epithelial interlamellar region although their presence has been demonstrated in the secondary epithelium (Avella *et al.*, 1987). Chloride cells exist in two forms in fresh water teleosts; α - and β - chloride cells and are found in fresh water adapted teleosts such as the euryhaline guppy *Lebistes reticulatus*, stenohaline gudgeon *Gobio gobio* and loach *Cobitis taenia* (Pisam *et al.*, 1987, 1990). Transfer of these fish from fresh to saline water induces the transformation of the α -chloride cells to the type present in fully adapted marine fish and the degeneration of the β - chloride cell (Pisam *et al.*, 1987).

The ultrastructure of the chloride cells is highly specialised for ionic transport (see figure 3:2). The cells (first observed by Keys and Willmer, 1932) are large, acidophillic, non mucous granular cells containing large quantities of mitochondria and an elaborate network of inter-connecting tubules of 60-80 nm diameter. This tubular reticulum is connected to the external environment as it is continuous with the basolateral membrane (Kanarky, 1986). The apex of each cell is firmly bound to the neighbouring pavement cells via a long and tight junctional apparatus (Laurent and Dannel, 1980).

FIGURE 3:2

Schematic Representation of a
Chloride Cell of a Marine Teleost



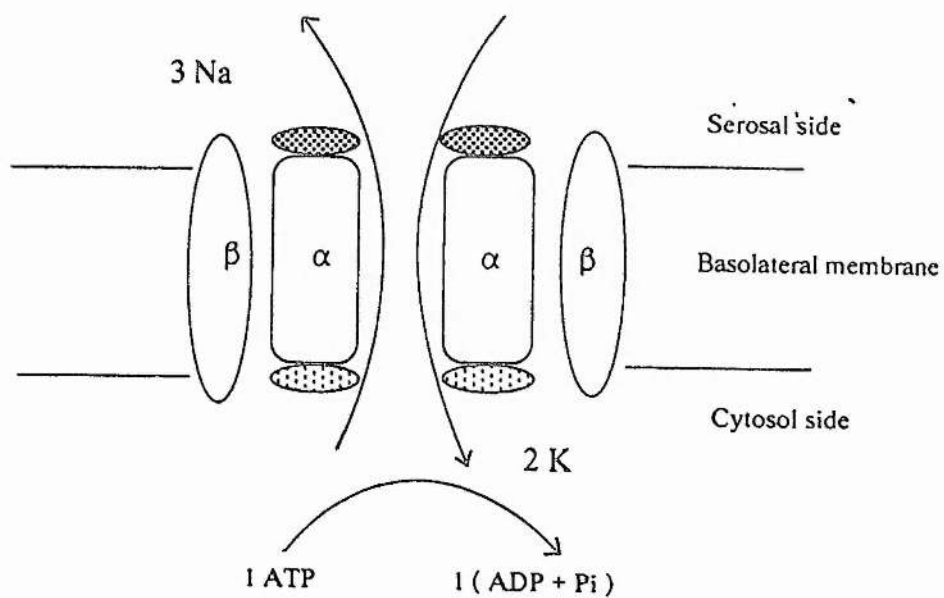
The cell's contact with the external medium is via depressions on the distal surface of the cell termed apical crypts (Maetz, 1971), the rest of the cell being entirely surrounded by epithelial cells (Philpott and Copeland, 1963). $\text{Na}^+\text{-K}^+\text{-ATPase}$ is an integral protein of the chloride cell plasma membrane and is responsible for the active Na^+ and K^+ exchange, against their concentration gradients. The composition of the $\text{Na}^+\text{-K}^+\text{-ATPase}$ molecule is shown in figure 3:3 and is of two heterologous polypeptide subunits spanning the entire basolateral membrane (Cantley, 1981). The α -subunit is catalytic and the β -subunit is glycosylated. All known functions of the molecule have, to date, been assigned to the α -subunit and the function of the β -subunit, although not clear, is thought to be essential to the functioning of the activity of the enzyme (Sweadner, 1989). This α -subunit is shown in figure 3:3 and has an ATP-binding site and a phosphorylation site on the cytoplasmic side, and on the extracellular side, a binding site for cardiac glycosides, such as ouabain, which inhibit the activity of the enzyme. This latter feature is employed in determining the enzyme activity.


There are two separate mechanisms to combat the osmoregulatory problems of fresh and salt water and are discussed below:

FIGURE 3:3

Schematic Representation of
 $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ Enzyme

(After Rankin and Davenport, 1981)



 Represents cardiac glycoside inhibitor sites.

 Represents ATP binding sites.

3.1.4 OSMOTIC AND IONIC REGULATION IN FRESH WATER TELEOSTS

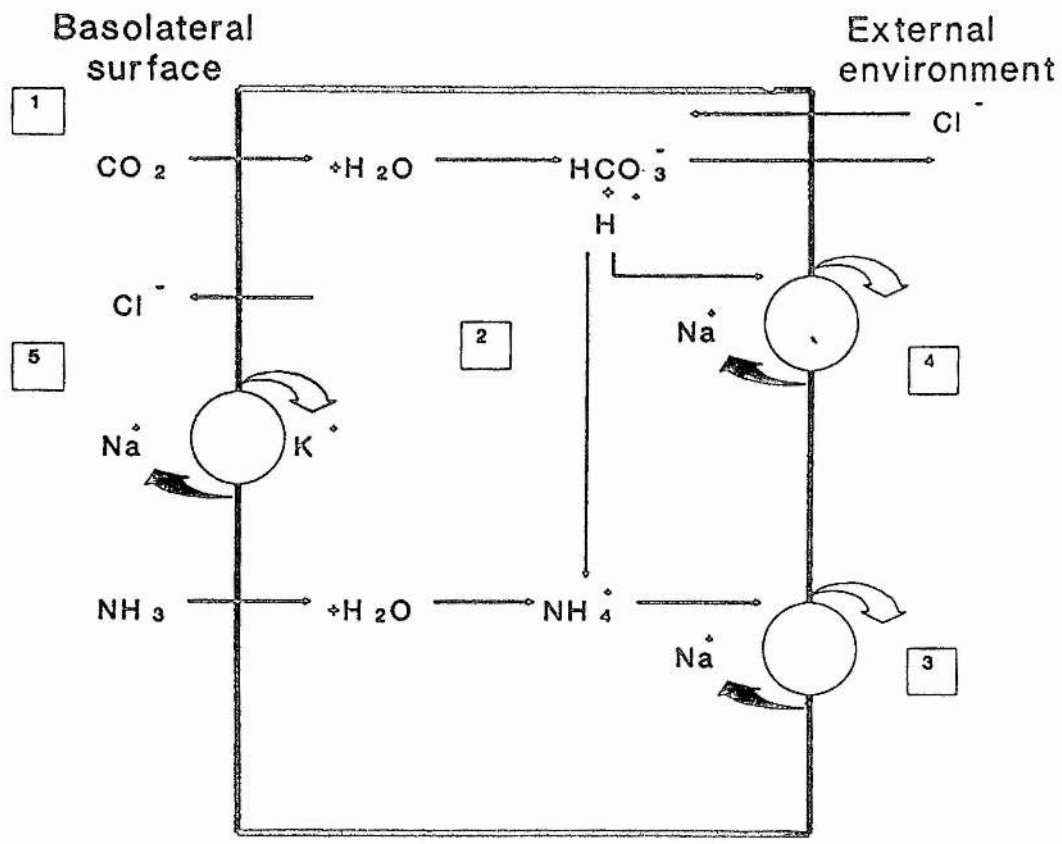
Due to the hypoosmotic environment provided by fresh water, there is an influx of water across the permeable membranes of the gills and pharynx. The kidney excretes copious quantities of hypotonic urine at 0.15–0.42% body weight per hour (Eddy, 1981) and the glomerular filtration rate is high. Ions are lost by passive diffusion across the gills and to combat this Na^+ and Cl^- are actively taken up through the gills and gut wall. It was suggested by Krogh (1939) and later confirmed by Maetz and Garcia-Romeau (1964) that Na^+ is exchanged for NH_4^+ and Cl^- exchanged for HCO_3^- . Maetz (1972) later observed that Na^+ is also exchanged for H^+ . The -40 mV potential between the fresh water fish and its environment is considered to be sufficient to halt any Na^+ efflux. Chloride cells are found in fresh water fish mainly at the junction of the primary and secondary lamellae of the gills. There are less chloride cells in fresh water fish than salt water fish and their role is controversial but they may be responsible for actively transporting salts into the fish together with the respiratory pavement cells (Pisam *et al.*, 1989). The gills of fresh water fish contain Na^+/K^+ -ATPase (Sargent and Thomson, 1974) and an anion-stimulated enzyme Cl^-/K^+ -ATPase is possibly involved in the Cl^- exchange with HCO_3^- . Figure 3:4 shows some possible ion exchange routes in fresh water teleosts.

FIGURE 3:4

Possible Mechanisms for Ion Uptake in the Gill of Fresh Water Fish

CO₂ degradation by carbonic anhydrase provides bicarbonate ions for exchange with Cl⁻ (1). The H⁺ from HCO₃⁻ breakdown can combine NH₃ (2) to produce NH₄⁺ ions which may be exchanged with Na⁺ (3). The alternatives are Na⁺-H⁺ exchange (4) or electrogenic H⁺ transport with passive flux of Na⁺ (not shown). The Na⁺ crosses the basolateral membrane via a Na⁺-K⁺ exchange pump with accompanying passive Cl⁻ transport.

(After Sainsbury, 1992)



The osmoregulatory function of the gut is well established in sea water adapted teleosts (see section 3.1.5) but is debated in fresh water adapted fish. Kirsch *et al.* (1984) reported a minimal role for the gut in freshwater however Baldisserotto and Mimura (1994) found $\text{Na}^+\text{-K}^+\text{-ATPase}$ in the posterior gut of *Anguilla anguilla* along with $\text{Cl}^-/\text{HCO}_3^-$, $\text{Na}^+\text{-K}^+\text{-Cl}^-$ and Na^+/H^+ transport proteins indicating transport activity by ion exchange and active transport. Unlike its homologue in tetrapods, the urinary bladder is more than just a storage organ. In three species of fresh water adapted flounder it has been shown to affect the final composition of the urine by reducing its permeability to water and increasing active ion absorption capacity (Hirano *et al.*, 1973). Stenohaline freshwater adapted fish have urinary bladders which are impermeable to water (Hirano *et al.*, 1973).

3.1.5 OSMOTIC AND IONIC REGULATION IN MARINE TELEOSTS

In marine fish due to the high environmental osmolality, water is lost by osmosis and salts enter the fish by passive diffusion via the gills. The skin is generally thicker in marine teleosts than in fresh water teleosts. There is little urine produced consisting of only 0.05% body weight per hour because the glomerular filtration rate is considerably lower than in fresh water fish. As teleost fish do not possess an intermediate concentrating segment in the nephrons of the kidney, the maximal urine concentration is isosmotic. Marine fish must drink considerable quantities of sea water, 0.5% body weight per hour,

to compensate for this water loss (Smith, 1930a; Perrott *et al.*, 1992).

Monovalent ions are excreted through the gills via the chloride cells which are abundant in the primary epithelium. Upon transfer to sea water, the number of these cells proliferates as do the number of accessory cells. Accessory cells are smaller than chloride and pavement cells (Laurent and Dunnell, 1980) and are linked to chloride cells via single strand leaky short junctions. Transepithelial movement of Na^+ may occur via these junctions whereas Cl^- is actively transported across the chloride cells. The enzyme $\text{Na}^+-\text{K}^+-\text{ATPase}$, responsible for the transport of ions across the gill epithelia, is localized in the basolateral membrane (Silva *et al.*, 1977). Na^+ is transported into the tubular reticulum in exchange for K^+ thus establishing a Na^+ concentration gradient with reduced Na^+ concentrations in the cytoplasm (Kanarky, 1986). Energy is derived from the movement of Na^+ down the electrochemical gradient which is maintained by the hydrolysis of ATP to ADP and inorganic phosphate by $\text{Na}^+-\text{K}^+-\text{ATPase}$. Three Na^+ ions are translocated to the extracellular compartment coupled to two K^+ ions transported to the intracellular compartment per molecule of ATP hydrolysed (figure 3:3) (Schuurmans Stekhoven and Bonting, 1981). Forrest *et al.* (1973) found a correlation between Na^+ efflux and $\text{Na}^+-\text{K}^+-\text{ATPase}$ activity in salt water adapted eels. Secretion of Cl^- is inhibited by ouabain which indicates the involvement of $\text{Na}^+-\text{K}^+-\text{ATPase}$ (Marshall, 1981) and is also inhibited by the absence of Na^+ (Degan and Zadunaisky, 1980). Cl^- is coupled to the Na^+

gradient by an Na-K-Cl co-transporter moving Cl^- into the cytoplasm from where it is extruded via a "CFTR" like chloride channel in the apical crypt (Kanarky, 1986). This transepithelial transport of Cl^- creates a potential gradient allowing the Na^+ to exit through the leaky chloride cell or chloride accessory junctions (Philpott, 1980). Figure 3:5 shows a model for salt secretion across the chloride cell in sea water adapted teleosts.

Entry of divalent ions into the body fluid is small as there is minimal gut absorption and these ions therefore pass out in the faeces. The small quantity of divalent ions that are absorbed (mainly Mg^{2+} and SO_4^{2-}) are excreted in the urine (Roberts, 1989). The intestine as an osmoregulatory organ is well studied. Na^+ and Cl^- are absorbed during the uptake of water (Kirsch *et al.*, 1984). This is coupled to the Na^+ - K^+ - Cl^- co-transport system in many species including the Japanese eel *Anguilla japonica* (Ando and Subramanyam, 1990).

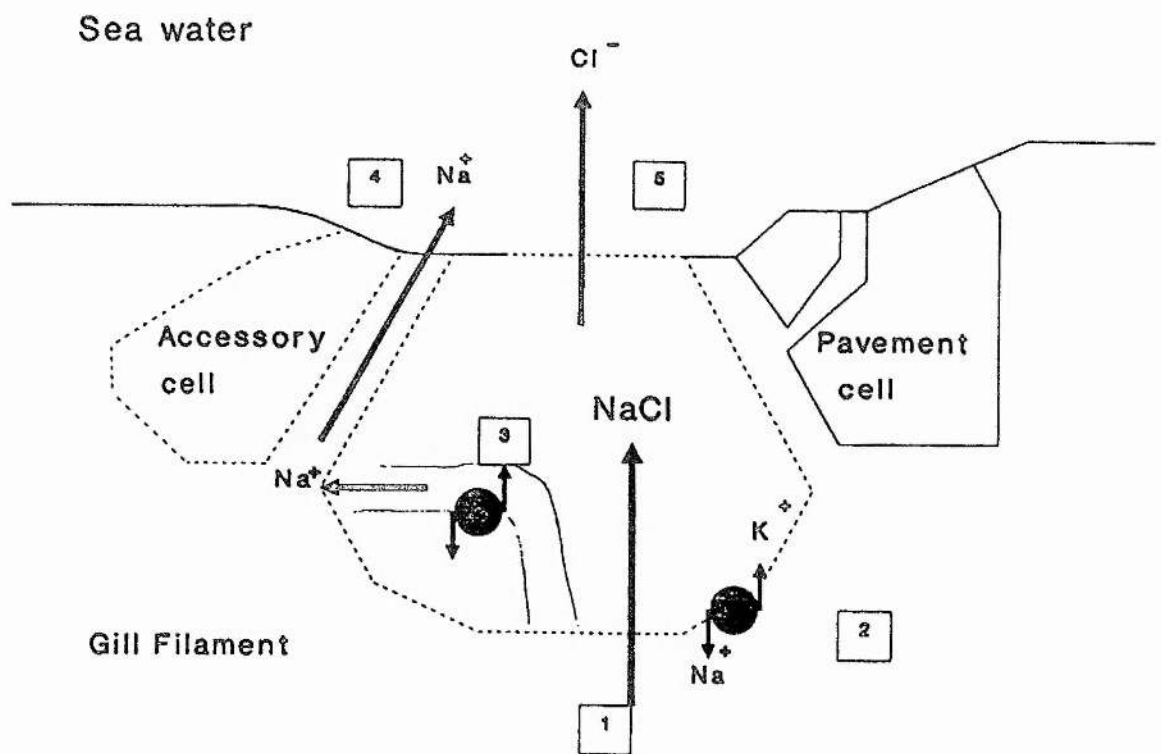
The urinary bladder in sea water adapted fish has a high permeability to water and a low rate of ion absorption. Euryhaline fish of sea water origin appear to be capable of adapting their urinary bladder to that of a fresh water fish when transferred to fresh water although this does not occur for fresh water species transferred to sea water (Hirano *et al.*, 1973).

FIGURE 3:5

Route and Mechanism of Salt Extrusion Across the Gill in a Sea Water Fish

NaCl enters the chloride cell (1) down a concentration gradient maintained by a basolateral $\text{Na}^+\text{-K}^+$ exchange pump (2). The Na^+ is either recycled or enters the tubular system (3). From here the Na^+ is extruded via a paracellular route (4). The Cl^- is removed across the apical membrane by an electrogenic pump (5).

(After Sainsbury, 1992)



3.1.6 OSMOTIC AND IONIC REGULATION IN EURYHALINE TELEOSTS

Many species of fish, such as salmon, trout, eels, mullet and flounder, are capable of migrating between fresh and salt water. Much work has been performed on smoltification in salmonids, as the adaptation process can be methodically monitored. Ionic and osmotic regulation is as described above and there is an increase in the number of chloride cells as the fish enters salt water together with an increase in cortisol activity (see below, section 3.2.1).

3.1.7 PHYSIOLOGICAL EFFECTS OF DIFFERENT SALINE ENVIRONMENTS

Much work has been performed on the effects of environmental salinities on the maintenance of blood composition of fish. Most work has been performed on full strength sea water (34-35 ppt) or fresh water but very little work has been performed on intermediate salinities such as isosmotic (11-12 ppt) environments.

Dutil *et al.* (1987) followed the migration of maturing American eels *Anguilla rostrata* from fresh to sea water and measured serum Na^+ , Cl^- and K^+ concentrations, the number of branchial chloride cells and $\text{Na}^+-\text{K}^+-\text{ATPase}$ activity. It was found that serum ion concentrations and osmolality were slightly lower in eels in fresh water than salt water. Eels transferred from fresh to sea water increased the number of chloride cells within 96 hours,

however, the number did not decrease when eels from salt water were transferred to fresh water. Gill $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity increased as the fish migrated towards sea water and fresh water eels kept in sea water for 96 hours showed an increase in gill $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity. Sea water adapted eels kept in fresh water for the same time span showed reduced $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity relative to sea water fish. Diseased eels had a much lower serum ion concentration and osmolality but if transferred to sea water these parameters were restored to near normal conditions, suggesting the eels were encountering problems of ion retention or volume control in fresh water. Thus eels entering salt water adapt by increasing the number of chloride cells and $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity.

Changes in metabolism have also been reported, Soengas *et al.* (1991) used salinities of 12 ppt to stimulate metabolic changes in rainbow trout, *Oncorhynchus mykiss*, when fish of low $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity (in a non smoltification period) were transferred from fresh water to a salinity of 12 ppt to simulate adaptation to sea water. A few hours after transfer, reductions in the levels of liver glucose and glycogen were observed probably due to the observed increase in liver glycogen phosphorylase activity linked to a decrease in glycogen synthetase activity. This is the same situation observed when salmon smolts are transferred to the sea and Woo *et al.* (1978) found a decrease in liver glycogen and body lipids when *Oncorhynchus kisutch* and *O. tshawytscha* smolts were transferred to sea water. Fessler and Wagner (1969) have also observed a decrease in lipid reserves in steelhead trout

transferred to sea water. It is speculated that energy reserves are mobilised to support the energetic demands of the osmotic challenges presented to fish transferring from fresh to salt water (Soengas *et al.*, 1991).

Many hormones are involved in the adaptation of fish to sea water. These are briefly summarised below.

3.2 HORMONES INVOLVED IN OSMOTIC AND IONIC REGULATION

3.2.1 CORTISOL

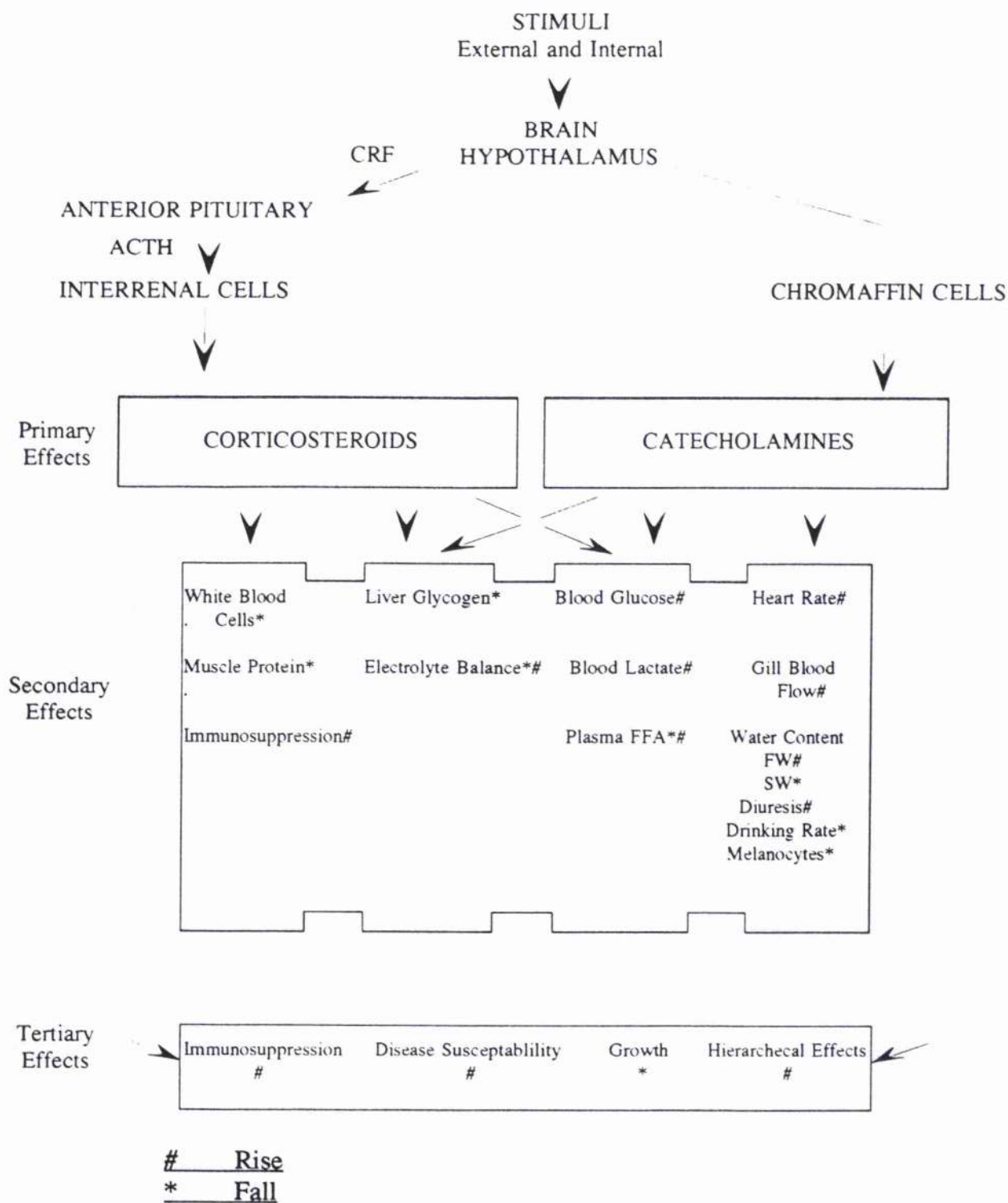
Several corticosteroids have been discovered in the blood of fish including; cortisol, cortisone, corticosterone, 11-deoxycortisol and 20B-dihydrocortisone (Donaldson, 1981., Mazeaud and Mazeaud, 1981). The main corticosteroid secreted by the interrenal tissue in fresh and salt water fish is cortisol (Sandor *et al.*, 1967) and is considered here. Much attention has been given to cortisol because of its secretion in fish subjected to stress (Tort *et al.*, 1995) (see chapter 4) and it is considered to be an indicator of chronic stress in fish and to a lesser extent prolonged acute stress (Avella *et al.*, 1991., Pickering, 1992).

The release of cortisol by the interrenal tissue is as a result of stimulation of the hypothalamus-pituitary-interrenal (HPI) axis (Donaldson, 1981) where adrenocorticotrophin (ACTH) is released from the pituitary (rostral pars distalis) which is itself regulated by corticotrophic releasing factor (CRF) from the nucleus lateralis tuberis (NLT) and the nucleus preopticus of the hypothalamus (Fryer and Peter, 1977) (see figure 3:6). There is evidence of other hormones being involved (Schreck *et al.*, 1989).

FIGURE 3:6

Diagrammatic Representation of
the Interaction of Primary,
Secondary and Tertiary Consequences
of Stress

(Modified After Mazeaud *et al.*, 1977)



Modified after Mazeaud et al., 1977

Corticosteroids belong to a group of lipids having a basic cycloperhydrophenanthrene nucleus of three six-membered carbon rings and one five membered ring joined by common sides. The steroid groups are subdivided according to the number of carbon atoms they contain and are derivatives of cholesterol compounds. There are the corticosteroids with 21 carbon atoms (21C), androgens (19C) and oestrogens (18C) (see figure 3:7) the latter two being sex steroids and are mainly effective in reproduction. Corticosteroids are divided into glucocorticoids (involved in metabolism), such as cortisol and corticosterone, and mineralcorticoids (involved in osmoregulation) e.g. aldosterone in mammals, but in many cases corticosteroids exhibit both mineralo and glucocorticoid activity.

The role of cortisol in teleosts has been investigated in a number of fish and perhaps the most studied groups are the anguillid eels; *Anguilla anguilla*, *A. rostrata* and *A. japonica* (Bentley, 1982, Norris, 1985).

Cortisol, in close association with prolactin, is involved in the regulation of water and electrolyte balance (Specker and Schreck, 1982), with the exact function varying between taxonomic groups. It is particularly important in euryhaline fish migrating from fresh to salt water where its interaction with prolactin is particularly evident (Bentley, 1982). Gill membranes are more permeable, osmotically and diffusionally, in fresh water than in marine teleosts (Isaia, 1984) and form an important target for cortisol which stimulates active ion transport in fresh and salt

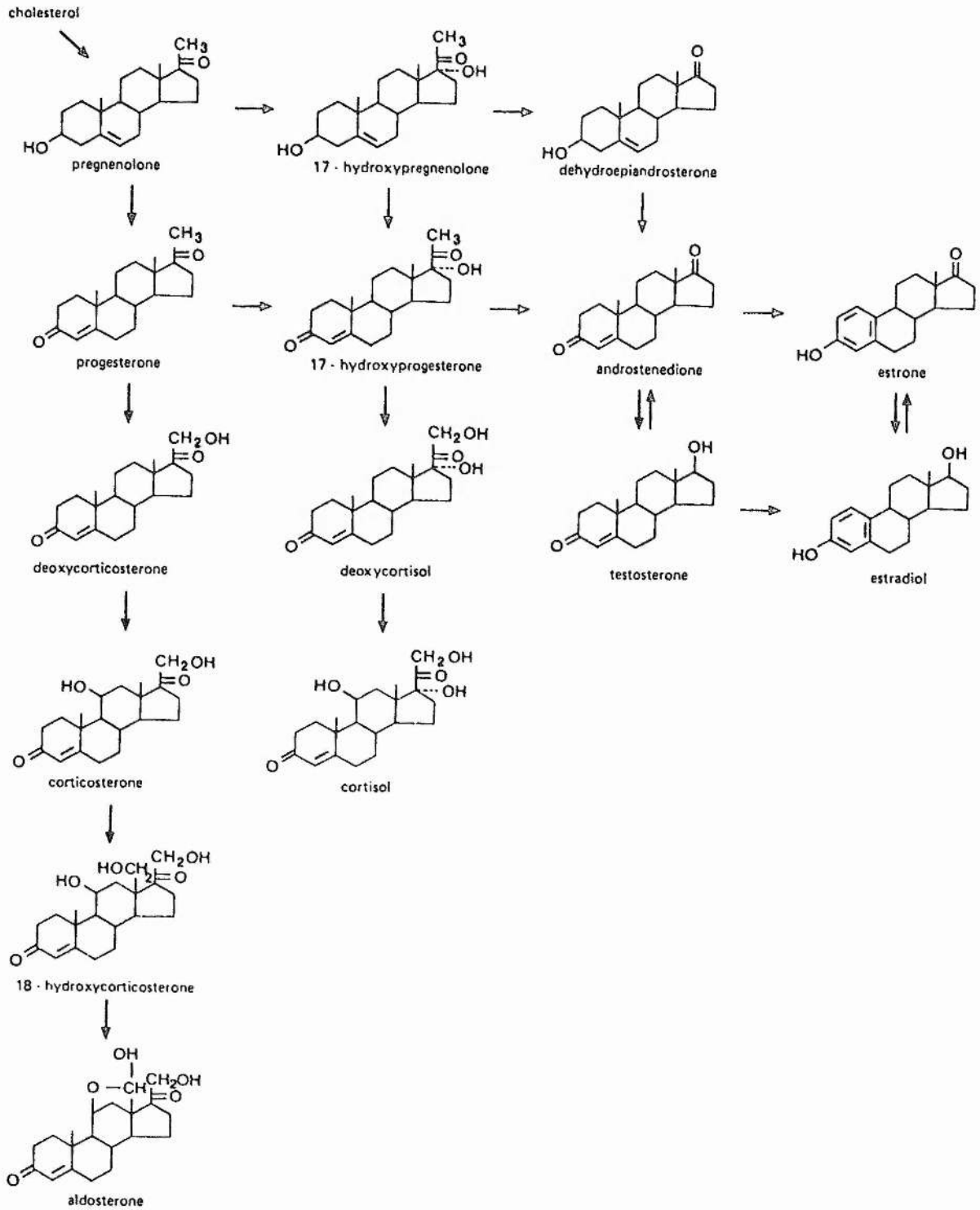
FIGURE 3:7

Interrelationships and Formation
of the Steroid Hormones

MINERALOCORTICIDS

GLUCOCORTICIDS

SEX HORMONES



water fish. Ion fluxes are 50-60 times greater in sea water adapted eels than in fresh water adapted eels due to the effect of imbibing sea water and the chloride cells rapidly excreting excess salt across the gill epithelial membranes (Bentley, 1982; Norris, 1985). Cortisol increases $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity in gill filaments and intestinal mucosa of fresh water adapted American eels *Anguilla rostrata*. This response to cortisol is comparable with that of the increase in $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity of fresh water adapted eels directly transferred to salt water (Epstein *et al.*, 1971). Similar results have been reported in migratory salmonids; fresh water adapted pre and post coho salmon smolts show an increase in $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity in the gill filaments of coho salmon. This increased activity is induced by cortisol in preparation for seaward migration (McCormick and Bern, 1989). The $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity is located mainly in the basolateral membrane of the chloride cell (Karnaky *et al.*, 1976). The increase in $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity can also be induced by the injection of cortisol (Laurent and Perry, 1990) in rainbow trout *Oncorhynchus mykiss*. This increased activity is due to an increase in the quantity of the enzyme $\text{Na}^+\text{-K}^+\text{-ATPase}$ (McCormick and Bern, 1989) required for adaptation to salt water conditions (Sargent and Thompson, 1974).

Normally there is little difference in the plasma concentrations of cortisol in fresh water and sea water adapted eels. However, upon transfer from fresh to sea water there is a sharp rise in blood cortisol concentrations persists for several days and then returns to normal levels (Forrest *et al.*, 1973). This evidence

suggests that cortisol is promoting sea water adaptation by initiating ionic transport mechanisms.

Na^+ accumulates in hypophysectomized or adrenalectomized fresh water adapted eels introduced to sea water apparently due to a reduction in blood cortisol (Norris, 1985). In fresh water, these fish have a reduced plasma Na^+ , reduced urine flow and reduced Na^+ reabsorption these observations further implicate cortisol in the regulation of water and ionic balance. Treatment with cortisol or ACTH does not completely restore the balance, probably due to the combined action of cortisol and prolactin (Norris, 1985).

Cortisol administered to coho salmon has been shown to induce chloride cell proliferation and differentiation resulting in the secretion of Cl^- upon introduction of the fish to the sea (Foskett *et al.*, 1983).

Injection of cortisol into fresh water adapted fish induces the hyperosmotic response of chloride cells. Since this response is also induced by introducing fish to sea water, cortisol has been termed the "sea water hormone".

Other hormones involved in osmoregulation are now considered briefly.

3.2.2 PROLACTIN

Prolactin (PRL) is a peptide hormone produced by the adenohypophysis of teleost fish and is closely related to Growth Hormone. PRL is mainly involved in salt transportation and osmotic permeability in skin, gills, opercular membrane, kidney, urinary bladder and intestine (Foskett *et al.*, 1983, Brown and Brown, 1987).

Hypophysectomized killifish *Fundulus heteroclitus* will not survive in fresh water, a situation that can be corrected by the administration of ovine PRL (Pickford and Phillips, 1959). PRL stimulates $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ activity in the gills and gut and hence ionic transport. In hypophysectomized fish this activity is reduced and Na^+ concentration in the blood plasma is consequently decreased (Brown and Brown, 1987). Increased renal sodium loss and branchial osmotic permeability together with reduced electrolytes in the plasma were observed in some but not all hypophysectomized fish, and similar results were obtained by the use of PRL blocking agents (Norris, 1985). The plasma of fresh water fish is reported to contain ten times the PRL activity of sea water adapted fish (Norris, 1985).

Other effects of PRL include:

- 1) General reduction in the permeability of the fish
- 2) Increased skin mucus
- 3) Reduced branchial permeability to water and ions
- 4) Reduced permeability of skin epithelium
- 5) Inhibition of intestinal fluid and ion absorption.

- 6) Inhibition of water uptake in the kidney
- 7) Stimulation of active ion transport in the kidney and urinary bladder
- 8) Stimulation of $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity and hence active ion transport
- 9) Increased GFR acting as a diuretic.

(see review, Hirano, 1986)

PRL acts antagonistically to cortisol and is sometimes referred to as "fresh water hormone".

3.2.3 GROWTH HORMONE

Growth hormone (GH) is an adenohypophysial hormone which primarily stimulates somatic growth in mammals (Gorbman *et al.*, 1983). Smith (1956) was the first person to observe a positive effect of GH on the survival of sea water adapted brown trout *Salmo trutta*, and the hormone has been isolated and sequenced from several species of teleost fish (Kawauchi and Yasuda, 1989). GH is considered to have an osmoregulatory effect as both its blood concentration and its MCR increase when salmon smolts enter the sea (Hirano *et al.*, 1987). However, this is not the case in the eel *Anguilla anguilla* (Kishida and Hirano, 1988) and therefore GH is not considered particularly significant in the osmoregulation of anguillid eels. Concentrations of GH peaked two days after rainbow trout *Oncorhynchus mykiss* were transferred to

sea water, these levels were transient and returned to pre-transfer concentrations (Sakamoto *et al.*, 1990). Rainbow trout *Oncorhynchus mykiss* and chum salmon *Oncorhynchus keta* injected with homologous GH showed no increase in plasma Na^+ when transferred to sea water. This would be expected if GH had not been administered (Hasegawa *et al.*, 1987; Sakamoto *et al.*, 1990, 1991). This reaction is not, however, displayed in *Anguilla japonica* which can uniquely survive sea water even after hypophysectomy (Takei, 1993) whilst hypophysectomized coho salmon, *Oncorhynchus kisutch*, are unable to survive such a transfer (Nishioka *et al.*, 1987). The role of GH in osmoregulation is not universal and may be of more importance in salmonid species.

3.2.4 ARGININE VASOTOCIN

Arginine vasotocin (AVT) is a basic octopeptide found in the neurohypophysis. AVT injected in large quantities into teleost fish will cause an increase in blood pressure and diuresis whereas low doses appear to be anti diuretic (Henderson and Wales, 1974). Pang *et al.* (1983) eliminated the vascular effects of high blood pressure by maintaining a trout trunk preparation at constant pressure and discovered that AVT acted anti diuretically. It is likely that the diuretic effect observed when high concentrations of AVT are administered *in vivo*, is due to increased GFR due to an increase in blood pressure.

Recent evidence suggests that AVT may be more important as a hormone of fresh water adaptation. AVT is secreted when sea water adapted euryhaline teleosts are transferred to fresh water. Pituitary content of AVT in fresh water adapted flounder are far greater than the levels of AVT in the pituitary of the sea water adapted flounder (Balment *et al.* 1993).

There is some evidence to suggest that the gill epithelium is a direct target for AVT. AVT may therefore be involved in chloride cell proliferation in sea water adapted fish (Guibbolini and Lahiou, 1990).

3.2.5 RENIN ANGIOTENSIN SYSTEM

In teleosts renin, a proteolytic enzyme, is produced in the juxtaglomerular cells in the kidney and released into the blood where it converts renin substrate (angiotensin) to the biologically inactive angiotensin I (AI). AI is in turn converted into the biologically active octopeptide angiotensin II (AII) by a converting enzyme. This cascade of reactions is termed the renin angiotensin system (RAS).

The complete role of the RAS in teleosts is unknown but, by analogy to tetrapods, it has been suggested to regulate blood pressure, kidney function (GFR) and Na^+ regulation by acting on adrenocorticosteroids. The RAS may also interact with other hormones such as neurohypophysial peptides.

Renin, when injected into *Anguilla anguilla*, causes an increase in blood pressure, natriuresis and diuresis due to increased GFR (Bentley, 1982). This effect reflects the production of AII which may increase the blood pressure by acting as a vaso constrictor and by causing the release of catecholamines which also exert a pressor effect (Takei *et al.*, 1979; Balment and Carrick, 1985; Perrott *et al.*, 1992; Tiernay *et al.*, 1995).

In teleosts AII stimulates drinking. Drinking is inhibited by the administration of a converting enzyme inhibitor such as captopril which inhibits the conversion of AI into AII (Balment and Carrick, 1985, Tierney *et al.*, 1995). The RAS may be particularly important in sea water adaptation in euryhaline fish as it stimulates drinking perhaps due to a decreased blood volume. Decreased blood pressure due to haemorrhage leads to the release of renin which activates the RAS (Nishimura *et al.*, 1979). The AII target in the eel is the medulla oblongata whilst in mammals and birds it is the forebrain. The reason for this difference could be that the mammals and birds need to initiate a series of behavioral responses to feel thirst, seek water and drink, whereas fish need only drink (Takei *et al.*, 1979).

AII may stimulate the release of ACTH which in turn releases cortisol directly from the interrenal (Decourt and Lahlou, 1987) and promotes salt efflux across the gill. AII may also alter the renal excretion of fluids and ions.

The RAS is reviewed in more detail by Nishimura (1987).

3.2.6 CAUDAL NEUROSECRETORY SYSTEM

In teleosts there is a ventral swelling of the spinal cord near the posterior end containing axonal ends of Dahlgren cells which are neurosecretory in function (Bentley, 1982). This structure is termed the urophysis and its function is thought to be one of ion and water balance (Bern, 1985). Originally four active polypeptide extracts of the urophysis were extracted and termed the urotensins. Urotensins have been shown to act upon ion transport across the skin, opercular membrane, intestine and urinary bladder (Larson and Bern, 1987).

Urotensins I and II (UI and UII) have been extracted and sequenced (Bern, 1985, Larson and Bern, 1987). UI has 41 amino acids and a structure similar to corticotrophin releasing factor (CRF) in mammals and thus may have some effect on adrenocorticotrophic hormone (ACTH) release and, subsequently, cortisol release (Woo *et al.*, 1985). UII, a dodecapeptide, has some homology with somatostatin in mammals. There is therefore no structural similarity between the two urophysial hormones (Ichikawa *et al.*, 1986).

Larson and Bern (1987) surgically removed the urophysis but observed no osmoregulatory responses possibly due to the rapid regenerative properties of this structure and other systems compensating for the loss.

Urotensins may influence the synthesis and secretion of other hormones such as cortisol and indirectly affect the ion and water balance of the fish (Arnold-Reed and Balment, 1989). It has been proposed by Bern (1969) that the urotensins are involved in "fine tuning osmoregulation rather than playing a major role.

A detailed account of the urophysis is given by Larson and Bern (1987).

3.2.7 NATRIURETIC PEPTIDES

The natriuretic peptides are a family of related peptides produced by the heart and parts of the central nervous system. This family includes; Atrial Natriuretic Peptide (ANP), Brain Natriuretic Peptide (BNP), C-Type Natriuretic Peptide (CNP) and Ventricular Natriuretic Peptide (VNP). Three peptides with a high structural homology to ANP, VNP and CNP have been identified circulating in the blood of the eel *Anguilla japonica* (Takei, 1993). To date, ANP, the earliest of the peptides to be discovered, has been the subject of most research although this has involved the use of heterologous peptides and antibodies.

An osmotic challenge leads to an increase in the production and release of ANP. Utilising an RIA for mammalian ANP, Evans (1990) showed that plasma levels are higher in sea water adapted fish than in fresh water fish. ANP stimulates Cl^- secretion via the chloride cells in the gills and inhibits intestinal Na^+ uptake.

Evans (1990) suggested that the stimulus to this activity is the presence of salt rather than volume loading. Mammalian ANP stimulates vasodilation, diuresis and natriuresis in glomerular and aglomerular fish (Evans 1990). Using heterologous peptides, Arnold-Reed and Balment (1993) showed that ANP stimulates cortisol release. This suggests that ANP may be involved in sea water adaptation. In the first studies to utilise homologous peptides ANP and VNP inhibited drinking in fresh water and sea water adapted eels *Anguilla japonica* (Takei and Balment, 1993). In addition ANP inhibited Na^+ absorption in the gut of the sea water adapted eels and the potency of the effects of ANP was found to be 2-3 times that of other hormones known to have a similar effect (Takei and Balment, 1993). Furthermore ANP induced antidiuresis but not antinaturesis in fresh water adapted eel (Takei and Balment, 1993). ANP is considered to be involved in sea water adaptation as it stimulates cortisol secretion (Arnold-Reed and Balment, 1991; Takei and Balment, 1993). It is possible that ANP and VNP have opposite effects in osmoregulation as observed in GH and PRL as previously described (Takei, 1993). It would therefore appear that ANP plays a complex role in osmoregulation but this may relate to the use of heterologous peptides in physiological studies.

3.2.8 THYROID HORMONES

The thyroid gland, and the hormones it secretes (T_3 and T_4), is responsible for many actions on biological systems compared to

any other gland (Gorbman *et al.*, 1983). Thyroid hormones are also influential on the osmoregulatory processes in teleosts especially in the parr-smolt process of adaptation to sea water (Grau, 1987) although the complete involvement is by no means fully understood (Dickhoff and Sullivan, 1987).

3.2.9 VASOACTIVE INTESTINAL PEPTIDE

Vasoactive intestinal peptide (VIP) is a neuropeptide located within the peripheral nerve terminals of the intestinal walls. Little is known of its action but it has been shown to stimulate Cl^- secretion in the opercular epithelium of sea water adapted tilapia *Sarotherodon mossambicus* (Foskett *et al.*, 1982) and winter flounder *Pseudopleuronectes americanus*. The action of VIP is mediated by a rise in cyclic AMP levels (Collie and Hirano, 1987). VIP inhibited the transport of Na^+ and water by the anterior intestine of both sea water and fresh water adapted tilapia *Sarotherodon mossambicus* (Mainoya and Bern, 1984). VIP may therefore be involved in sea water adaption. However more work on other species will be necessary before the function of VIP is fully established.

3.3 MATERIALS AND METHODS

3.3.1 GROWTH RATE EXPERIMENT

The first experiment was designed to investigate the long-term effects of increased water salinity on growth rates and stress of eels. Eels, initially weighing 5-10 g were obtained from Western Aquaculture, transported by road to Scotland and maintained at 23°C. in two recirculating aquaculture systems as described in chapter 2. After an initial period of six weeks during which the biofilters were developing, fish were graded into two identical populations consisting of three size categories:

Small	(12 g average weight)	-	1485 fish
Medium	(24 g average weight)	-	447 fish
Large	(48 g average weight)	-	458 fish

This initial differential growth is typical of commercial eel aquaculture (Seymour, 1984). The large fish are removed since this accelerates growth of the smaller fish (Droogendijk pers. comm.). After a two week adaptation period two experimental systems were established each containing small, medium and large populations:

FW System - fish maintained in freshwater

SW System - fish maintained at 11-12 parts per thousand (ppt) saline water (common salt NaCl was added to the water

over a period of six days to avoid osmotic shock)

During the operation of each system, water was added to compensate for evaporative water losses only and salinity was monitored constantly using a salinity refractometer (Aquafauna Biomarine). Water quality was determined throughout the experiment on a daily basis. pH, NH_4 , NO_2 , NO_3 , dissolved O_2 , air temperature and water temperature were measured and recorded (for detail see chapter 2).

Fish were fed standard commercial salmon diets (BP Nutrition). The size of the crumb/pellet fed was dependant on the size of fish (see table 3:1). Feeding was on a twice-daily basis to satiation by lowering the food on a feeding tray; the tray with any surplus food was removed when the fish showed no further interest. The composition of the diet is given in table 3:1.

Fish weights were determined at the beginning of the experiment and thereafter at 4-5 weekly intervals by weighing the total fish population thus avoiding errors due to variation in sampling which is a particular problem in eel aquaculture. In addition, fish weights were determined for individual fish removed for experimental purposes (groups of 20-25) again at approximately 4-5 week intervals. These time intervals were chosen as initial experiments showed that more frequent removal of fish for weighing had a detrimental effect on appetite and subsequent growth rates. This effect was almost certainly due to the acute stress induced by the procedure (see chapter 5).

TABLE 3:1

Size and Composition of BP Nutrition
Fry Diets Used in Study

SIZE AND COMPOSITION OF BP NUTRITION FRY DIETS

Description:

	Crumb Diameter	Fish Average Weight
Fry 02	1.1-1.5 mm	< 10 g
Fry 03	1.5-2.3 mm	10-30 g

Composition:

Protein	55%
Oil	15%
Carbohydrate	11%
Gross Energy	20.82 MJ/kg
Digestible Energy	17.79 MJ/kg

SIZE AND COMPOSITION OF BP NUTRITION "ROYALE" DIETS

Description:

	Pellet Diameter	Fish Average Weight
Freshwater Smolt 1	2.0 mm	30-75 g
Freshwater Smolt 2	2.5 mm	> 75 g

Composition:

Protein	52%
Oil	15%
Carbohydrate	13%
Gross Energy	20.45 MJ/kg
Digestible Energy	17.29 MJ/kg

Fish removed for experimental purposes were used in the calculation of cortisol metabolic clearance rates (MCR) (see chapter 4) and blood samples were taken for plasma composition analysis.

3.3.2 STOCKING DENSITY EXPERIMENTS

The primary purpose of the stocking density experiments (Experiment 1 below) was to assess the long term effects of increased stocking densities on the on growing of eels in both freshwater and 12 ppt saline water. Once this had been determined the second objective was to determine the maximum densities at which growth still occurs and which are appropriate for eel culture. The experimental conditions in this second series of experiments (Experiments 1 and 2 below) were identical to the initial freshwater versus saline water growth rate experiment (section 3.3.1).

Experiment 1

Eels between 24-67 g (freshwater adapted) and 24-67 g (saline water adapted) were used. In order to avoid sampling errors the complete population of fish was weighed and the average weight gain was normalised to represent average weight gain per 43 days taking account of mortalities. Stocking densities investigated were between 50-130 kg/m³ (freshwater) and 50-165 kg/m³ (saline water). The increase in stocking density was achieved by periodically adding fish to the experimental tanks.

Experiment 2

As the effects of stocking density become more critical with increased body weights of fish, growth rates of fish above 100 g, average weight 108.1 g (freshwater) (n = 806) and average weight 110.8 g (saline water) (n = 786), were examined. Equal stocking densities of 130 kg/m³ were established for both freshwater and saline water fish based on the data obtained from Experiment 1. Growth of the fish was assessed by two methods:

Method 1

Random samples of the two populations were taken (on days 0, 52 and 137 of the experiment) and individual lengths and weights recorded.

The number of fish measured at each time point were:

Day 0 - Fresh water n = 66	Salt water n = 74
Day 52 - Fresh water n = 157	Salt water n = 157
Day 137 - Fresh water n = 163	Salt water n = 163

The condition factor (CF) was calculated according to the formula:

$$CF = W/L^3 \times 100$$

Where:

W = weight

L = length

Blood (for plasma analyses) was taken from the fish into heparinized tubes by decapitation and plasma was separated by spinning in a centrifuge at 6,500 rpm for 1 minute. The plasma was separated from above the erythrocytes by dropping pipette and placed in another tube and frozen until analyzed.

Method 2

The complete population was weighed (on days 0, 41, 72, 103, 134, 164 and 208) as in the growth rate experiment thus avoiding sampling errors. Average weight of each population was then calculated by dividing the biomass by the total number of fish surviving.

3.3.3 RADIOIMMUNOASSAY OF CORTISOL

Cortisol was extracted from 50 μ l plasma samples by means of Sep Pak C18 cartridges using a modification of procedures previously described by Hofreiter *et al.* (1983) and Armour *et al.* (1993). The plasma was made up to 1 ml through the addition of 0.9% saline. The Sep Paks were conditioned with 5 ml methanol, washed with 20 ml distilled water and samples applied at a rate of 0.5 ml/min. The column was then washed with 2 ml of 20% acetone, followed by 2 ml distilled water and cortisol eluted at a rate of 0.25 ml/min in 5 ml methanol. The methanol was evaporated off, the steroid reconstituted in 1 ml phosphosaline buffer ($\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 39.0 mM; NaHPO_4 , 61.3 mM; NaCl , 154.0 mM). Recovery values of 98% for the extraction of cortisol from plasma were

obtained, utilising recovery of ^3H cortisol.

Cortisol plasma concentrations were determined by radioimmunoassay utilising a specific antibody (Scottish Antibody Production Unit, SAPU) according to the techniques of Waring *et al.* (1992). 0.5 ml of antibody (1 : 1,000 dilution) were added to either 250 μl of reconstituted, ethanol-extracted plasma samples or cortisol standards ranging from 20–0.156 ng/ml (both made up in phosphosaline buffer). The mixture was vortexed, stored at room temperature for 30 min and then approximately 10,000 cpm ^3H -cortisol was added. Tubes were incubated overnight at 4°C. The bound and free radioactivity were separated by addition of charcoal and spun at 4°C. for 15 min. 1 ml of scintillation fluid was added to 100 μl of the supernatant and each tube counted for 10 min on a liquid scintillation counter (Packard Tri-Carb 2000). Using this technique inter-assay variation was calculated to be $2.45 \pm 0.69\%$ (n=14) and intra-assay variation was assessed at $4.23 \pm 0.25\%$ (n=14).

3.3.4 PLASMA ANALYSIS

Plasma osmolality was determined by freezing point depression (Roebeling Osmometer) plasma chloride concentration by titration (Corning 925 chloride meter). Initial attempts to determine red blood cell and lymphocyte cell number indicated a large variation between individual fish in the same experimental group. It was therefore decided to measure haematocrit alone by micro-

centrifugation of whole blood in haematocrit tubes.

3.3.5 STATISTICAL TESTS

Data were analyzed by ANOVA with subsequently either Bonferroni or Dunnet post test. Where only two experimental groups existed they were analyzed with Student's t test (unpaired) or the alternative Welch t test.

3.4 RESULTS

3.4.1 GROWTH RATE EXPERIMENT

3.4.1a Growth Rates

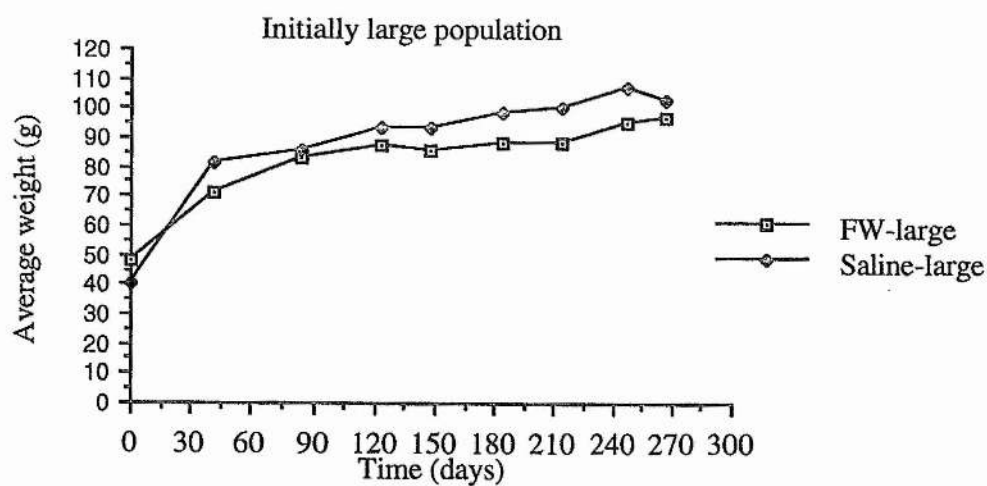
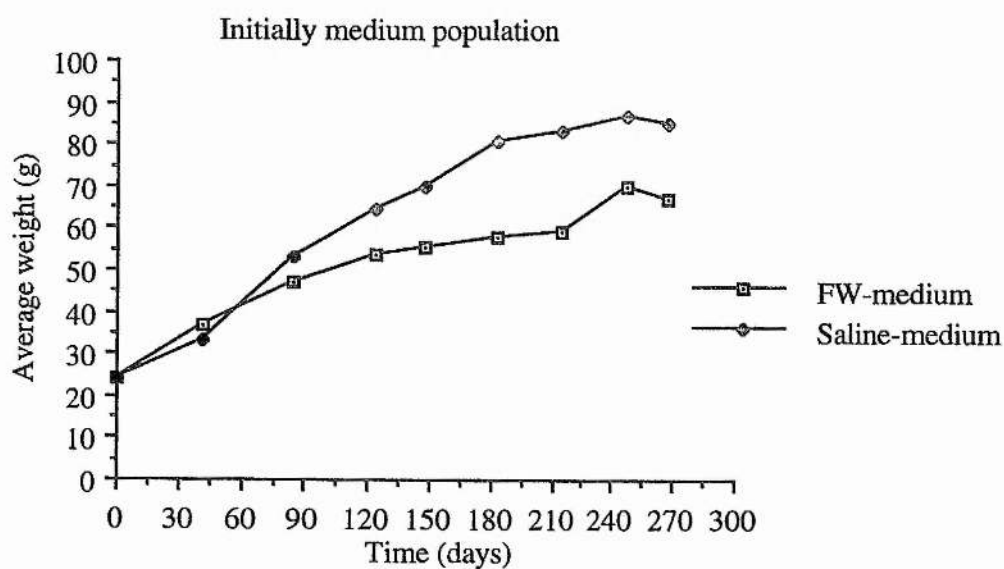
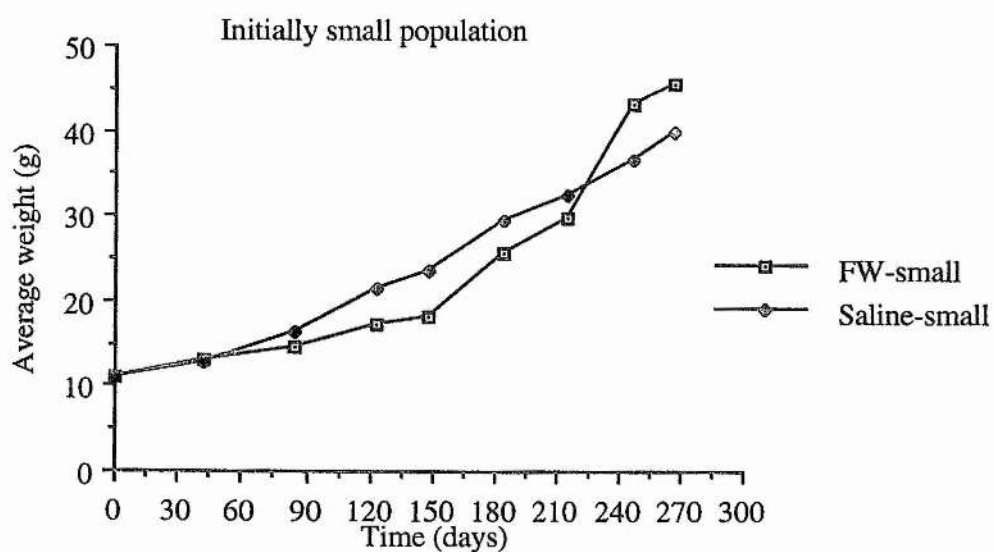
The growth rates of the small, medium and large populations are shown in figure 3:8. It is clear that in the medium and large populations of eels maintained in saline water, the growth rate is greater than in freshwater. ANOVA analysis demonstrated a significant difference between the growth curves ($p < 0.01$) of freshwater and saline water fish, and subsequent post-ANOVA test, utilising Bonferroni (protected) comparison, showed that from 125 days to the end of the experiment the body weight of saline water eels was significantly greater ($p < 0.01$) than for fresh water eels. Therefore there appears to be a "growth premium" in eels cultured in saline water. There was a similar result for large eels but not for fish in the initially small category (see below).

After 125 days there was an accidental loss of fish from the small population (29.39%) due to a leaking oxygen inlet (see section 2.3.2d). Due to this occurrence, the stocking density in the fresh water tank containing the small fish fell from 37.67 kg/m³ to 26.62 kg/m³, the small saline water fish remained at 44.8 kg/m³. After 220 days there was a further accidental loss of fish (25.89%) in the freshwater tank containing the small fish due to a combination of pump and alarm failure.

FIGURE 3:8

Increase in Average Body Weight
for Initially Small, Medium and
Large Populations in Either Fresh
Water or Saline Water (12 ppt)

Average weight was obtained by weighing the total
biomass and dividing by the total number of fish
in each group.



The corresponding slight increase in the growth rate of the fresh water adapted fish was probably due to the resulting reduction in stocking density from 44.98 kg/m³ to 33.33 kg/m³, the saline water fish remained at 70.25 kg/m³. This demonstrates the importance of stocking density for growth rate in the experimental groups.

Figures 3:9 a-c show the final weight distributions in the three initial (small, medium and large) populations. As the populations were derived from splitting the intake fish after grading into 2 identical populations (see section 3.3.1) (one fresh water and one saline water) it is assumed that the proportion of small, medium and large fish within the small, medium and large populations are also identical. These histograms demonstrate that in the medium and large populations there was an increased proportion of large fish in the final population.

3.4.1b Mortality rates

With the exception of the unavoidable losses of fish, as detailed above due to the equipment failure, the normal monthly mortality was less than 1% in both fresh water and saline water groups. These rates are similar to those obtained in commercial systems.

3.4.1c Plasma composition

The plasma osmolality and plasma chloride concentrations for the medium population groups are shown in figure 3:10 and table 3:2.

FIGURE 3:9

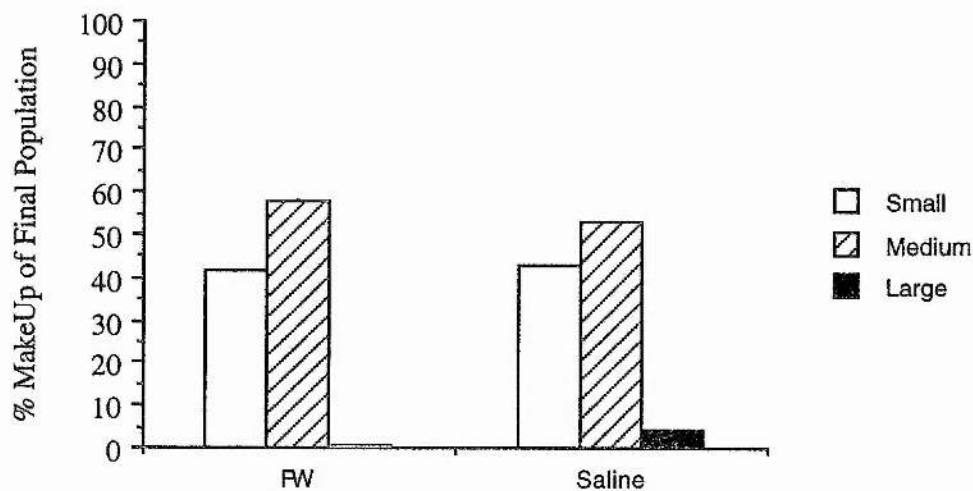
Histogram to Show the Development
of Initially Small, Medium and Large
Populations in Fresh Water and Saline
Water (12 ppt)

The initial populations were graded into small, medium and large populations at the start of the growth experiments. Each of the final populations were graded into small, medium and large populations and the make up of these final populations is displayed in the histograms.

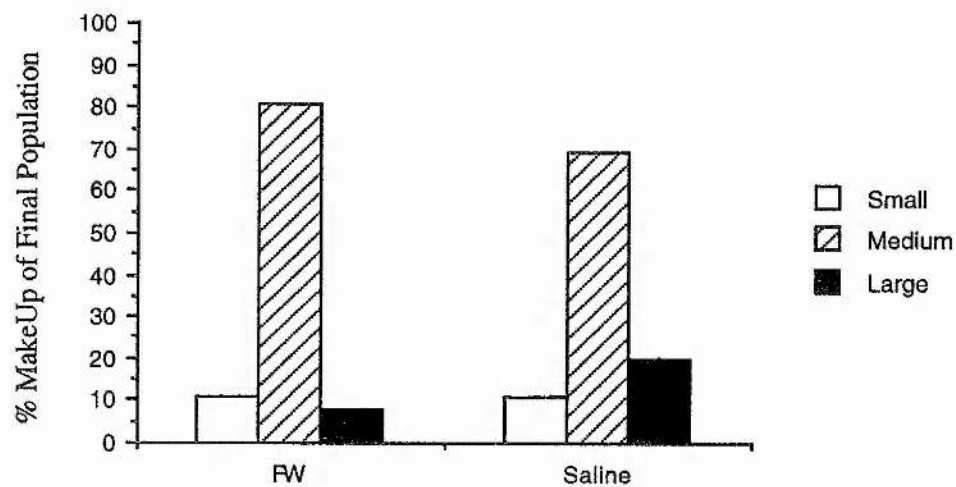
Average Weights Were:

Initial populations	Small	11 g
	Medium	24 g
	Large	48 g
Final populations	Small	22 g
	Medium	90 g
	Large	142 g

Initial Mean Body Weight (11g)



Initial Mean Body Weight (24g)



Initial Mean Body Weight (48g)

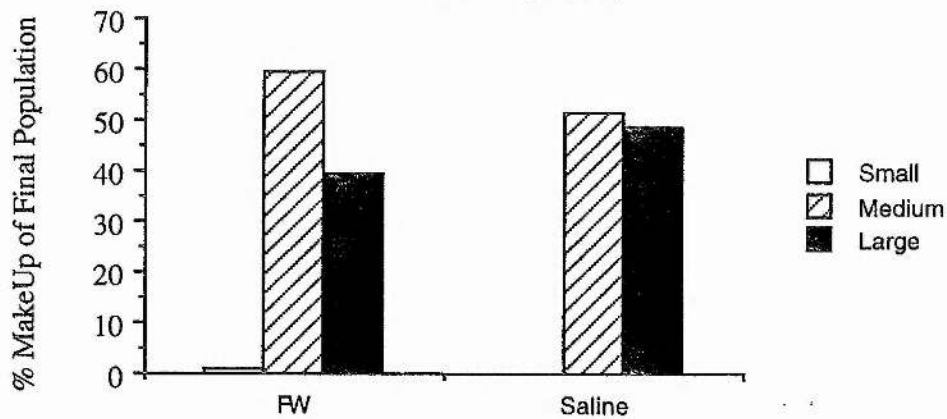


FIGURE 3:10

Plasma Osmolality and Chloride
Concentration During Growth in
Either Fresh Water or Saline Water
(12 ppt) for Eels in the Medium
Population

Results are means \pm SEM of at least 17 fish
in each group

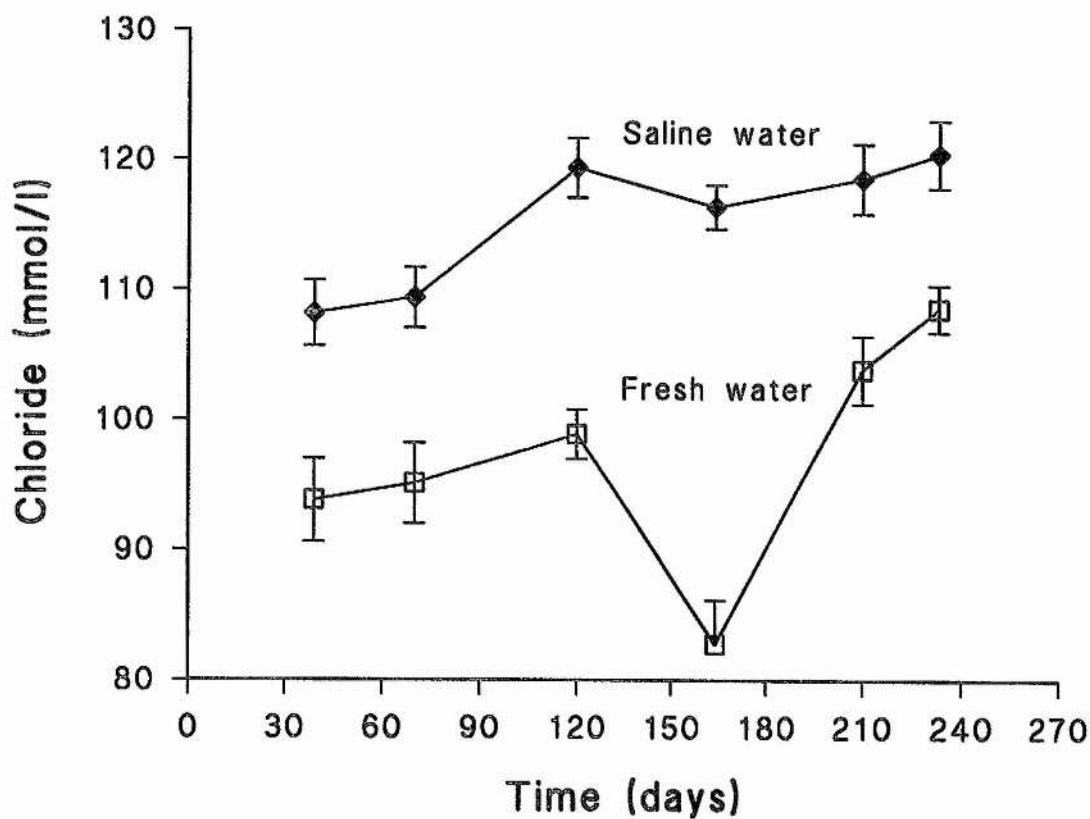
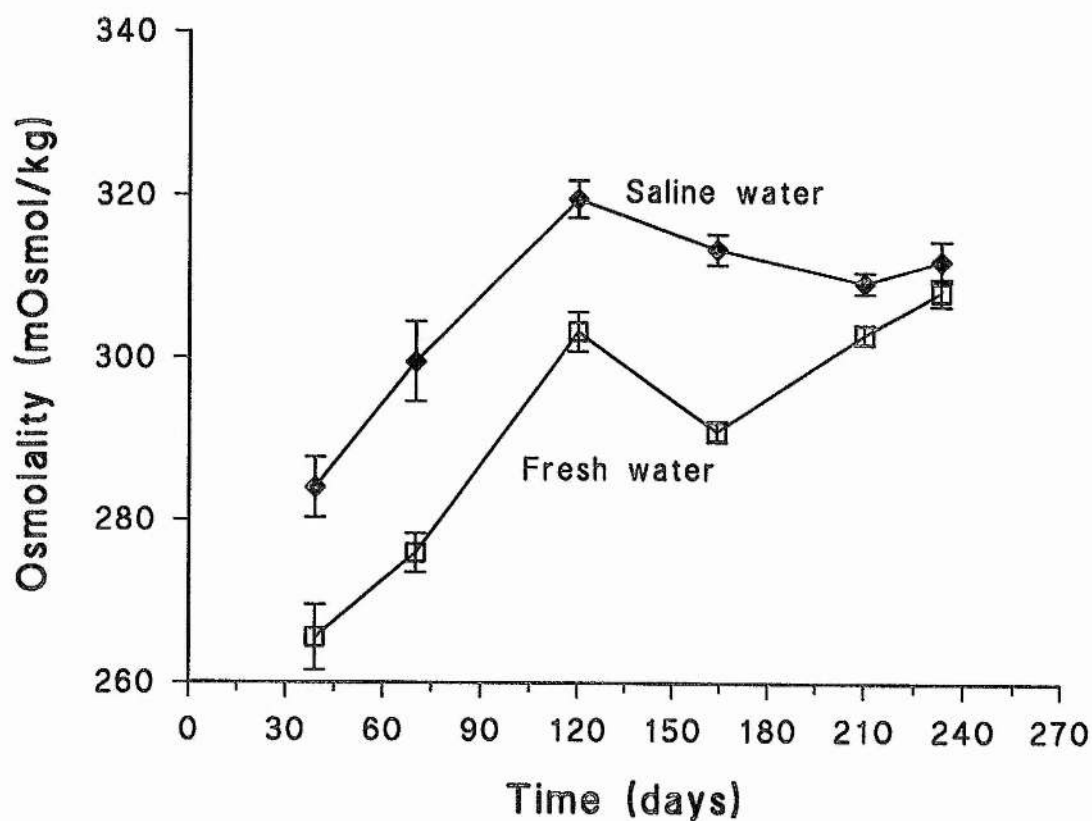


TABLE 3:2

Plasma Osmolality and Chloride
Concentration During Growth in
Either Fresh Water (FW) or Saline
Water (SLW) (12 ppt) for Eels in
the Medium Population

Results are means \pm SEM of at least 17 fish
in each group * indicates a significant difference
between saline fish values ($p < 0.05$) and those in
the fresh water group

Time (days)	Osmolality (mOsmol/kg)		Chloride (mmol/l)	
	FW	SLW	FW	SLW
39	265.8 ± 4.0	284.0 ± 3.7*	93.8 ± 3.2	108.2 ± 2.5*
70	275.9 ± 2.4	299.5 ± 4.9*	95.1 ± 3.1	109.4 ± 2.3*
120	303.2 ± 2.4	319.5 ± 2.3*	98.9 ± 1.9	119.4 ± 2.3*
164	290.9 ± 1.3	313.3 ± 1.9*	82.8 ± 3.4	116.4 ± 1.7*
210	302.9 ± 1.2	309.2 ± 1.3*	103.9 ± 2.6	118.6 ± 2.7*
233	308.1 ± 1.7	311.9 ± 2.5	108.6 ± 1.8	120.5 ± 2.6*

In both experimental groups the plasma osmolality increased from 39 to 120 days after which it maintained an approximately steady state. Plasma osmolality was consistently higher in the saline water group ($p < 0.002$). In both experimental groups, plasma chloride concentrations increased throughout the period monitored with the exception of one point in the fresh water group at 164 days where there is an unexplainable fall. With the exception of the final time point the saline water values were always significantly greater than the equivalent freshwater group ($p < 0.001$ in all cases). As previously recorded, red blood cell number was highly variable in eels of the same experimental group. This was reflected in haematocrit values which ranged from 25% to 50%. There was no significant difference in haematocrits between freshwater or saline water but in saline waters haematocrits were typically 3-4% higher than in freshwater.

The plasma cortisol concentrations (table 4:1, chapter 4) were low in both fresh and saline water groups and there were no significant differences between them.

3.4.2 STOCKING DENSITY EXPERIMENTS

3.4.2a Growth Rates

Experiment 1

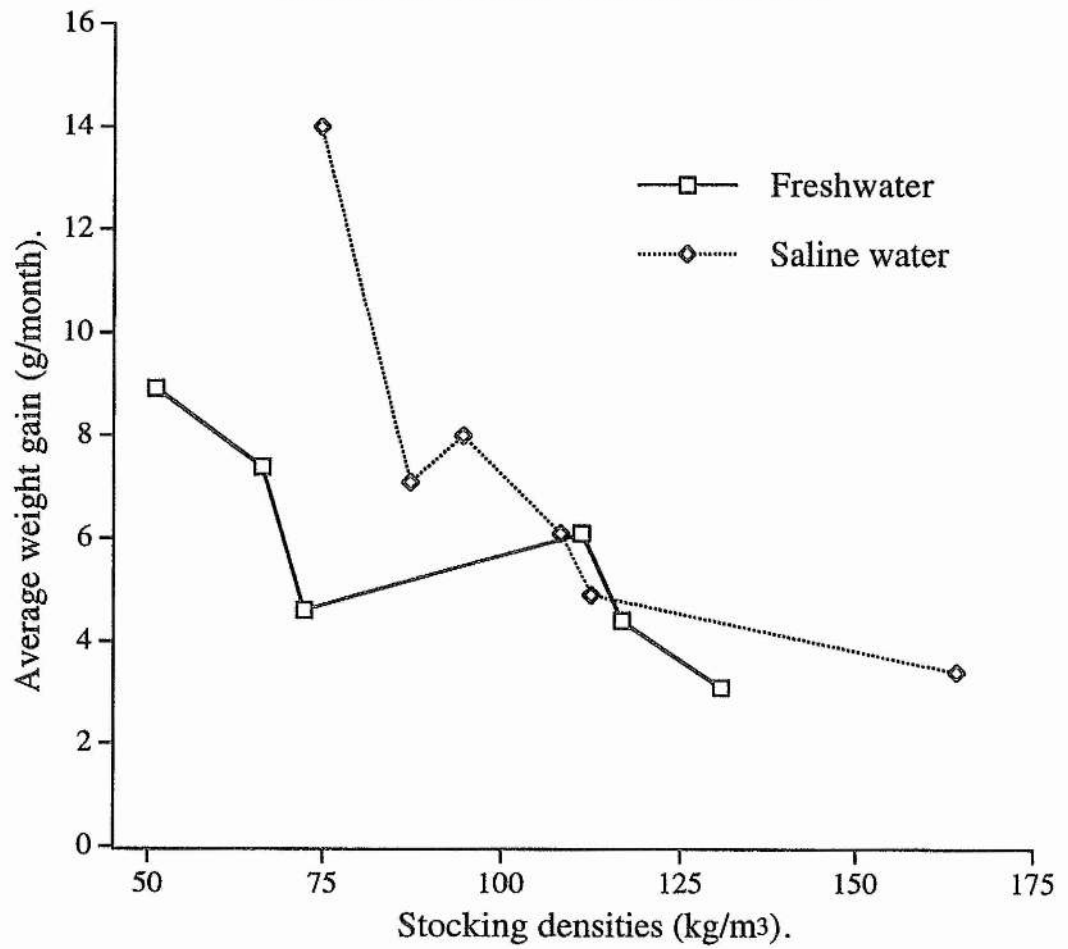
As expected, when stocking densities increased the relative growth rates of fish decreased (see figure 3:11).

FIGURE 3:11

Change in Average Weight Gain of
Fish in Fresh Water and Saline Water
(12 ppt) at Increasing Stocking
Densities

Average weight was obtained by weighing the total biomass and dividing this by the total number of fish in each group. This value was then subtracted from the previous average weight to show the gain

Change in average weight gain of fish in freshwater or saline water at increasing stocking densities.



However, in a closed warm water recirculating aquaculture system the volume of water is limited both by physical constraints and the increased costs associated with circulating and heating large volumes of water. Optimal economic stocking densities are therefore a compromise between maximal growth rates and practical considerations. The increase in growth rate of fish maintained in saline water, first demonstrated in the initial salinity trial (section 3.4.1a) is apparent throughout the range of stocking densities investigated (figure 3:11). It was therefore decided to conduct a prolonged experiment at 130 kg/m^3 in order to assess growth rates at close to the maximum stocking density at the two different salinities.

Experiment 2

Method 1

Table 3:3 indicates that there was no significant increase in the mean body weight of fresh water eels sampled 52 days and 137 days into the experiment. There was a significant increase in the body weights of saline water fish after 137 days even at this high stocking density. This suggests that in freshwater the maximum stocking density had been exceeded and this was confirmed by a reduction in condition factor throughout the experiment (table 3:3). The condition factor determination in saline water was complicated by the effect of the contaminated salt but there appeared to be no sustained decrease.

TABLE 3:3

Average Weight, Plasma Osmolality,
Plasma Chloride and Condition Factor
of Fish Grown at an Initial Stocking
Density of 130 kg/m³ in Either Fresh
Water (FW) or Saline Water (SLW) (12 ppt)

Results are means +/- SEM of at least 12 fish
in each group. * indicates a significant difference
in saline fish values ($p < 0.05$) from those in the
fresh water group

Day	Average Weight (g)		Plasma Osmolality (mOsm/kg)		Plasma chloride (mmol/L)		Condition Factor	
	FW	SLW	FW	SLW	FW	SLW	FW	SLW
0	108.0 ± 3.1	108.9 ± 3.0	294 ± 8	312 ± 8	134 ± 2	141 ± 2*	0.2	0.19
52	110.3 ± 1.6	110.1 ± 2.6	288 ± 2	303 ± 3*	139 ± 2	147 ± 3*	0.19	0.18
137	110.6 ± 2.5	123.1 ± 3.1*	288 ± 7	297 ± 4	133 ± 8	147 ± 3	0.19	0.19

Method 2

Figure 3:12 shows that in freshwater at a stocking density of 130 kg/m³ there was no significant increase in the total weight of the population. However, the saline groups continue to grow during the same period, although the results are affected by an increase in mortalities that occurred, particularly between 41 and 72 days (3.9% per 31 days). This was traced to a contaminated batch of salt delivered from a different commercial supplier. When salt from the original supplier was substituted (on day 86) the increased growth rate in saline water or a "saline premium" was re-established (figure 3:12) and mortalities began to fall (1.9% days 72-103 ie per 31 days).

Over the 208 day experiment, the average weight gain in the fresh water fish was 1.2 g and 14 g in the saline water group.

3.4.2b Mortality rates

Mortalities were complicated by the contaminated batch of salt (see above, Experiment 2) but were less than 1% per month when not affected by the contaminated salt. Raising the stocking densities to 130 kg/m³ did not, therefore, affect the mortality rates.

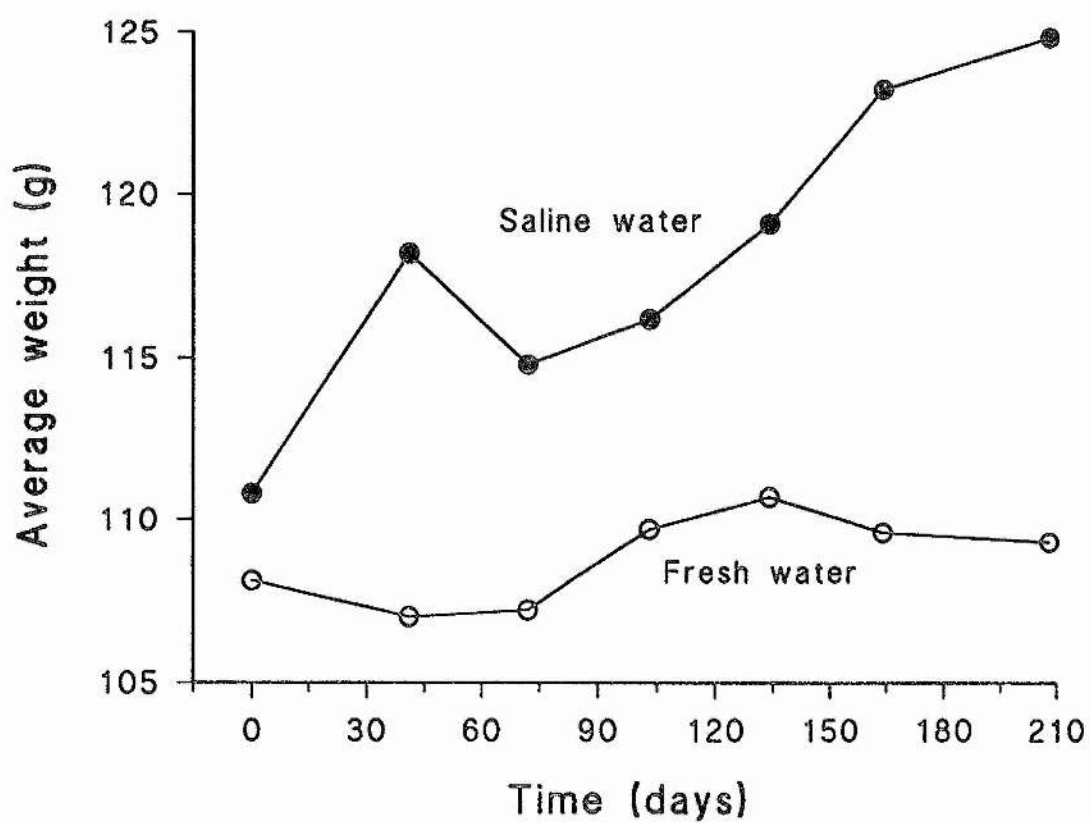
3.4.2c Plasma composition

Plasma osmolality was elevated in the saline water fish in comparison to the fresh water group on day 52 ($p = 0.0004$) but no such elevation was apparent on days 0 and 137 (table 3:3).

FIGURE 3:12

Change in Average Weight With Time
of Fish Grown in Fresh Water and
Saline Water (12 ppt) at a Stocking
Density of 130 kg/m³

Average weight was obtained by weighing the total
biomass and dividing by the total number of fish
in each group.



Plasma chloride concentrations were significantly higher in saline water fish for the first 52 days ($p < 0.05$) in comparison to the fresh water group (table 3:3). It is particularly interesting that plasma cortisol concentrations were significantly elevated ($p < 0.01$) in freshwater fish stocked at 130 kg/m³ but not in saline water (table 3:4) this is further discussed in section 4.5.2 in chapter 4.

TABLE 3:4

Plasma Concentrations of Cortisol,
in Fish Grown at an Initial Stocking
Density of 130 kg/m³ in Either Fresh
Water (FW) or Saline Water (SLW) (12 ppt)

Results are means +/- SEM of at least 12 fish
in each group. * indicates a significant difference
in saline fish values ($p < 0.05$) from those in the
fresh water group

Plasma cortisol (ng/ml)		
Days	FW	SLW
0	36 ± 11	25 ± 4
52	44 ± 5	20 ± 3*
137	57 ± 7	31 ± 6*

3.5 DISCUSSION

3.5.1 GROWTH RATE EXPERIMENT

The long-term salinity experiments conducted at 12 ppt saline water were the first of this type in practical eel aquaculture. With the exception of salinity, the water parameters in both experimental groups were very similar.

One of the reasons for the large increase in growth rate of saline water fish is that fish in the 12 ppt salinity expended less energy on osmoregulation. Although energy budgets were not calculated in this study it was shown that saline water fish maintained their plasma osmolality significantly higher than the freshwater eels and in effect were isosmotic to their environment. This prevents the obligatory osmotic water gain and electrolyte loss of freshwater fish and the corresponding increase in energy required to osmoregulate. Eels in 12 ppt salinity had no requirement to drink and therefore no energy was used in drinking. Few equivalent studies have been performed although dilute salt solutions have been used to alleviate acute stress during fish transport (Hattingh *et al.*, 1975; Kutty *et al.*, 1980).

3.5.2 STOCKING DENSITY EXPERIMENTS

Optimal stocking density depends on many factors including environmental parameters such as water chemistry, temperature, flow rate, physical characteristics of the system e.g. through flow or recirculating, species and age of the fish. In this study, the water composition was maintained constant, with respect to ; ammonia, nitrite, nitrate, oxygen, pH, temperature (see section 2.3.2a-g), at all stocking densities in both fresh and saline waters. The observed results were therefore independent of water quality. It was interesting that the freshwater eels at 130 kg/m³ stocking density therefore exhibited chronically increased plasma cortisol concentration and poor growth performance. However the saline water fish did not show increased cortisol concentrations. In addition, saline water fish held at 130 kg\m³ maintained growth rates although these were lower than those observed at reduced stocking densities. Pickering and Stewart (1984) reported that brown trout subjected to severe crowding showed a prolonged elevation of plasma cortisol levels. The effects of cortisol as a stress hormone will be discussed fully in chapter 4.

This work suggests that it is possible to increase stocking densities for eels maintained in saline water compared to eels grown in fresh water. However, the optimal stocking density would need to be carefully assessed for each particular culture system. One possible detrimental effect of saline water at the highest stocking density (130 kg/m³) was revealed from histological

studies which indicated a reduced number of mucous cells in the skin and gills of eels in 12 ppt saline water (Mandich and Hazon unpubl.). This is a normal feature of adaptation to increased salinity (Dunnell and Laurent, 1984). The skin covering the head and tail regions of a small number of the saline water fish, approximately 5% of the total, at the highest stocking densities, exhibited reduced mucous covering and abrasions. This was most likely due to physical abrasion, particularly during feeding, and possibly aggressive behaviour.

There have been, to date, no other studies of eel aquaculture using water of 12 ppt salinity. The large increase in growth rate observed in saline water provides a clear indicator for improved commercial aquaculture techniques, and should lead to increased financial efficiency (see chapter 6).

CHAPTER 4

Chronic Stress

CHAPTER 4

CHRONIC STRESS

4.1 STRESS IN FISH

The reduction of stress in farmed fish is extremely important. Stress has an adverse effect on survival, growth, reproduction, fish health and it increases the susceptibility of fish stock to disease (Schreck, 1981; Pickering and Stewart, 1984; Flos *et al.*, 1990; Pickering, 1992). This susceptibility to disease is stress related and is convincingly demonstrated by the method which has been developed to detect Atlantic salmon, *Salmo salar*, carrying *Aeromonas salmonicida* (causative agent of furunculosis). The fish suspected of infection are stressed chemically by injection of a synthetic corticosteroid such as prednisolone acetate or thermally by increasing temperature. Stress applied in this way causes fish carrying the disease to express symptoms of the disease. Stress results in dramatic changes in the endocrine system and it has been suggested that the immune system in fish can be modulated by hormones circulating in the blood (Sunyer *et al.*, 1995). In support of this suggestion it has been demonstrated that immunosuppression can be induced by corticosteroids in rainbow trout *Oncorhynchus mykiss* (Anderson, 1982).

Figure 3:6 in chapter 3 summarizes the interaction of primary, secondary and tertiary consequences of stress in fish.

An exact definition of stress is difficult to make and it has been debated at length over many years. Selye (1950) describes it as, "the sum of all the physiological responses by which an animal tries to maintain or re-establish a normal metabolism in the face of a physical or chemical forces." Brett (1958) relates stress to fish disease and writes, "stress is a stage produced by an environmental factor which extends the adaptive responses of an animal beyond the normal range, or which disturbs the normal functioning to such an extent that the chances of survival are significantly reduced." Schreck (1981), states that, "stress may be considered as an environmental externality which reduces the ability or capacity of fish to maintain homeostasis or do, physiologically and behaviourally, those things they must perform to persist, grow and reproduce." Therefore the environment has a considerable influence on the fish, and changes in the environment may directly induce stress.

A variety of methods have been used in an attempt to quantify stress over the years. Monitoring the levels of hormones associated with stress may be useful but many different hormones are reported to change in fish as a result of stress. Levels of growth hormone are reduced after confinement or netting (Pickering *et al.*, 1991), as are levels of prolactin (Pottinger *et al.*, pers. commun.). Thermal shock increases the levels of MSH and endorphin in the brown trout pituitary (Sumpter *et al.*, 1985). The pituitary-thyroid axis is also sensitive to acute and chronic stressors (Osborn and Simpson, 1974). Perhaps the most widely used methods are the changes in plasma concentration of

corticosteroids together with glucose in the plasma in fish (Schreck, 1981; Wedemeyer and McLeay, 1981).

Certain changes in response to environmental stress have been termed the general adaption syndrome (GAS) (Roberts, 1989) and have been classified into three categories: Primary (alarm reaction), secondary (resistance) and tertiary (exhaustion), (see discussion section 4.6 and figure 3.6 in chapter 3 for a more detailed account).

There is evidence to suggest that chronic stress such as overcrowding may cause increased plasma concentration of plasma cortisol (Pickering and Stewart, 1984; Pickering and Pottinger, 1989) and experimentally cortisol has been shown to suppress somatic growth (Peters and Schwarzer, 1985; Barton *et al.*, 1987). Further evidence suggests that the kinetics of cortisol secretion and clearance may be important in chronic stress (Redding *et al.*, 1984).

Stress can be classified in fish farming terms as either "chronic" or "acute". This classification is helpful when assessing the causes of stress on the farm and ways of reducing it. In this chapter only chronic stress will be considered as acute stress will be considered in chapter 5.

4.2 CHRONIC STRESS

Chronic stress is common in the aquaculture industry and common sources are listed below:

4.2.1 STOCKING DENSITIES

There is no doubt that high stocking densities can produce increased plasma concentrations of cortisol (Pickering and Stewart, 1984; Pickering and Pottinger, 1989) and suppress growth (see chapter 3). It is well known that high stocking densities induce stress and they are frequently used in experimental studies to induce stress. Barton and Schreck (1987) used stocking densities of 200 kg/m³ to induce stress in juvenile chinook salmon and reported that the plasma cortisol concentrations were elevated. Cortisol levels rose within the first 24 hours in juvenile chinook salmon exposed to a density of 800 fish/m³ and recovered within 48 hours (Strange *et al.*, 1977). The time to recover fully increased directly with the increasing density.

4.2.2 WATER QUALITY

Section 2.1.3 describes in detail the effects of water poor quality on recirculation systems. Some examples of how water quality may induce chronic stress are (all from Wagner, 1987):

- 1) Channel catfish exposed to a total ammonia nitrogen concentration of 100 mg/l showed raised cortisol concentrations which decreased after 24 hours as the fish began to adapt.
- 2) Nitrite concentrations of 5 mg/l increased cortisol levels in channel catfish which steadily increased during the first 24 hours.
- 3) Juvenile rainbow trout maintained at a pH of 4.7-5.7 for 5 days showed a small increase in cortisol level compared with fish at pH 6.6 but when handled for 30 seconds showed twice the level of cortisol compared to that of the control fish at pH 6.6.
- 4) Copper at 60-120 µg/l lead to a stress response in juvenile coho salmon.

4.2.3 TERRITORIAL BEHAVIOUR

Hierarchies can develop in crowded fish tanks with the subdominant fish becoming chronically stressed (Ejike and Schreck, 1980). The subdominant fish show external signs of stress namely; increased sensitivity to disturbance, inappetence, dark colour, inability to maintain their station in the water, inactivity at the surface and in corners and ragged fins and general appearance. Such-sub dominant fish are poor competitors for food. Above certain stocking densities this hierarchy tends to break down (Seymour, 1984).

4.2.4 DISEASE

Much work has been published on the subject of disease in fish. Stressed fish are susceptible to disease; for example rainbow trout injected with cortisol show increased susceptibility to disease (Robertson *et al.*, 1963., Kent and Hedrick, 1987). Unfortunately, the majority of treatments for diseases exert some degree of stress on the fish. The relationship between stress and disease is discussed in more detail in section 4.3.

4.2.5 MATURITY

Hormonal changes in sexually mature fish are the main causes of stress characterized by an increase in 17-hydroxycorticosteroids in the blood produced by an increased activity of the pituitary and interrenal glands. In such cases the pituitary later degenerates and the interrenal becomes hyperplastic (Robertson and Wexler, 1962.). Spawning salmonids, in the wild, are not only under corticosteroid induced stress but are also undergoing the effects of starvation, travel exhaustion and osmotic changes and frequently succumb to infections caused by fungi and bacteria which normally inhabit the skin and gut. These microorganisms are prevented from becoming pathogenic by the immune system but in fish suffering from exhaustion they are relatively unopposed and multiply to become pathogenic.

4.3 CORTISOL

4.3.1 ROLE OF CORTISOL IN CHRONIC STRESS

One of the objectives of this study was to assess stress in the eel *Anguilla anguilla* by measuring plasma cortisol concentrations and metabolic clearance rates (MCR). Elevated plasma cortisol levels, as a result of chronic environmental stress such as increased water temperature, pollution, poor water quality, social interaction and confinement, have been shown to decrease the survival rate of fish by increasing their susceptibility to disease (Hunter *et al.*, 1980., Peters *et al.*, 1984). Experimental cortisol implants in rainbow trout have induced an increase in mortality rates as a result of disease (Pickering and Pottinger, 1989). This effect appears to result from suppression of the immune system by cortisol (Tripp *et al.*, 1987; Maule *et al.*, 1989). Cortisol treated rainbow trout showed decreased growth rates (Barton *et al.*, 1987). In addition, growth rates decreased in handled trout suggesting that a stress-induced rise in blood cortisol levels was responsible (Peters and Schwarzer, 1985). Reproductive processes in sexually mature brown trout *Salmo trutta* were affected by cortisol which was administered by implant (Carragher *et al.*, 1989). The male fish showed reduced gonadotrophin levels in the pituitary which decreased the testosterone levels circulating in the blood and a subsequent significant reduction in the size of the testes was observed. Female fish treated in the same manner showed similar results

with less testosterone, oestradiol and vitellogenin in the blood leading to a decrease in the size of the ovary.

There is evidence to suggest that fish can acclimatise to stress and in some cases in which plasma cortisol concentrations were raised due to prolonged confinement, the cortisol levels may actually return to basal levels even though the stressor has not been removed (Pickering and Pottinger, 1989). Stocking density was found to have no effect on plasma cortisol concentrations in brown trout *Salmo trutta* (Pickering and Stewart, 1984), coho salmon *Oncorhynchus kisutch* (Schreck *et al.*, 1985), Atlantic salmon *Salmo salar* (Kjartansson *et al.*, 1988) and rainbow trout *Oncorhynchus mykiss* (Laidley and Leatherland, 1988). It has been speculated that fish held at high stocking densities may have a faster MCR (Schreck *et al.* 1985) and therefore have low cortisol concentrations even though the interrenal gland has increased activity. Trout held at high stocking densities did not increase plasma cortisol concentrations to the same degree as fish held at low stocking densities when subjected to handling and confinement (Pickering and Pottinger, 1987). These observations lend support to the conclusion that the interrenal tissue is either inactive or incapable of reacting to high stress levels (Pickering and Pottinger, 1987) or had an increased MCR. Vijayan and Leatherland (1990) concluded that cortisol concentrations were maintained at a low level in brook charr, *Salvenus fontinalis*, due to cortisol uptake by the liver. This appears to be an adaptation to high stocking densities where the damage caused by the long term effects of elevated plasma cortisol would

be limited. Adaptation to the long term effects of cortisol has been demonstrated in rainbow trout, *Oncorhynchus mykiss*, by Pottinger (1990) who showed that after long term exposure to cortisol the number of receptors in target tissues decreased.

The main effect of cortisol, in chronic stress situations, seems to be the mobilisation of energy reserves to meet the fish's increased energy demand (Chan and Woo, 1978., Vijayan *et al.*, 1991). Cortisol affects the fish's metabolism by acting as a glucoregulatory hormone (Reid and Perry, 1991; Maule *et al.*, 1989).

Clearly the plasma concentration of cortisol alone is not an accurate index of the corticosteroid status of the fish. To fully understand the effects of cortisol it is necessary to investigate the cortisol dynamics especially MCR and this subject will now be discussed.

4.3.2 CORTISOL CLEARANCE RATES

The concentration of a hormone in the blood is not an accurate indication of the production rate of that hormone as the hormone can be taken up rapidly at its target tissue, inactivated or excreted. Hormones, especially steroids such as cortisol, are often directly associated or bound to blood plasma proteins in two relationships; either a high capacity low affinity, or low capacity high affinity relationship. In either relationship there

is an equilibrium of free and bound hormone established. Only a relatively small amount of free hormone is available to the receptors in the target tissue from this buffered pool. There is much speculation about the purpose of this binding relationship, the most probable are that:

- 1) the hormone is removed from any enzymes in the blood which may deactivate the hormone
- 2) the protein bound to the hormone may actually add to the specificity of that hormone
- 3) binding may extend or moderate the hormone's action.

The available concentration of a hormone is a product of the equilibrium set up between the rates of production and catabolism rates, i.e. the production and removal rates must be equal for the hormone concentration in the plasma to be in a steady state. The term metabolic clearance rate (MCR) is defined as the volume of blood irreversibly cleared of a hormone per unit time (Tait *et al.*, 1962) and is calculated thus:

$$MCR = \frac{BPR}{C}$$

where, MCR = Metabolic clearance rate (ml/hour)

BPR = Blood production rate (µg/hour)

C = Endogenous steroid concentration (µg/ml)

4.3.3 DETERMINATION OF METABOLIC CLEARANCE RATE

4.3.3a By single injection

A single dose of radioactively labelled hormone (e.g. ^3H cortisol used in this study) is injected into the fish and the radioactivity in the blood is plotted as a function of time to display the clearance of the hormone. MCR is calculated thus:

$$\text{MCR} = \frac{\text{Ri}}{\text{A}}$$

where, Ri = Amount of radioactivity injected into the plasma (dpm)

A = Area under the radioactivity-time curve
(dpm/ml/hour)

4.3.3b By constant infusion

A steady state of radioactively labelled hormone is established in the blood by infusing the blood with a constant rate of the labelled hormone (Schulster *et al.* 1976). MCR is calculated thus:

$$\text{MCR} = \frac{\text{I}}{\text{Xc}}$$

where, I = Constant state of infusion (dpm/hour)

Xc = Steady state isotope concentration in the plasma (dpm/ml)

If required, the BPR can be calculated by:

$$\text{BPR} = \text{MCR} \times \text{C}$$

4.4 MATERIALS AND METHODS

4.4.1 EXPERIMENTAL CONDITIONS

The data generated in this section were from the same fish as used in chapter 3. Thus the details for the:

1) Growth Rate Experiment Populations and

2) Stocking Density Experiment Populations

are described in sections 3.3.1 and 3.3.2 of chapter 3.

4.4.2 RADIOIMMUNOASSAY OF CORTISOL

The procedure for the radioimmunoassay of cortisol is detailed in section 3.3.3, chapter 3.

4.4.3 DETERMINATION OF CORTISOL DYNAMICS

To determine the metabolic clearance rate (MCR), groups of eels were isolated from both freshwater and saline tanks. To determine MCR, eels were i.p. injected with 4 μCi (1, 2, 6, 7 ^{-3}H) cortisol (Amersham International) delivered in 50 μl of 2.5% ethanol: saline solution. Samples of six fish were killed at 1, 2, 5, 12 and 24 hour post-injection, weighed and blood taken by decapitation. Blood was collected in heparinized tubes. 50 μl of plasma was added to a scintillation cocktail (1 ml) and total

radioactivity determined by scintillation counting (Packard Tri-Carb 2000). Radioactivity was expressed as percentage of injected dose per ml plasma and individual fish were standardised to 100 g body weight. The MCR of ^3H -cortisol was determined by regression analysis using a semi-logarithmic plot of %injected dose/ml plasma/100 g body weight with time. The disappearance curve of ^3H -cortisol was bi-phasic (figure 4:1) with a so-called 'fast' and 'slow' component. The data were analyzed by the method of "curve-peeling" (Shipley and Clark, 1972) using the computer program Enzfiter, the general regression equation was:

$$C = Ae^{-\alpha t} + Be^{-\beta t}$$

C = percentage of injected dose

t = time

β = slope of slow component

B = intercept of slow component

α = slope of fast component

A = intercept of fast component

The distribution volume of ^3H -cortisol 'V' is calculated from $V = 100\%/A$ and the fractional turnover rate/hour 'k' is equal to α so that $\text{MCR} = V\text{k}$ (Higgs and Eales, 1976).

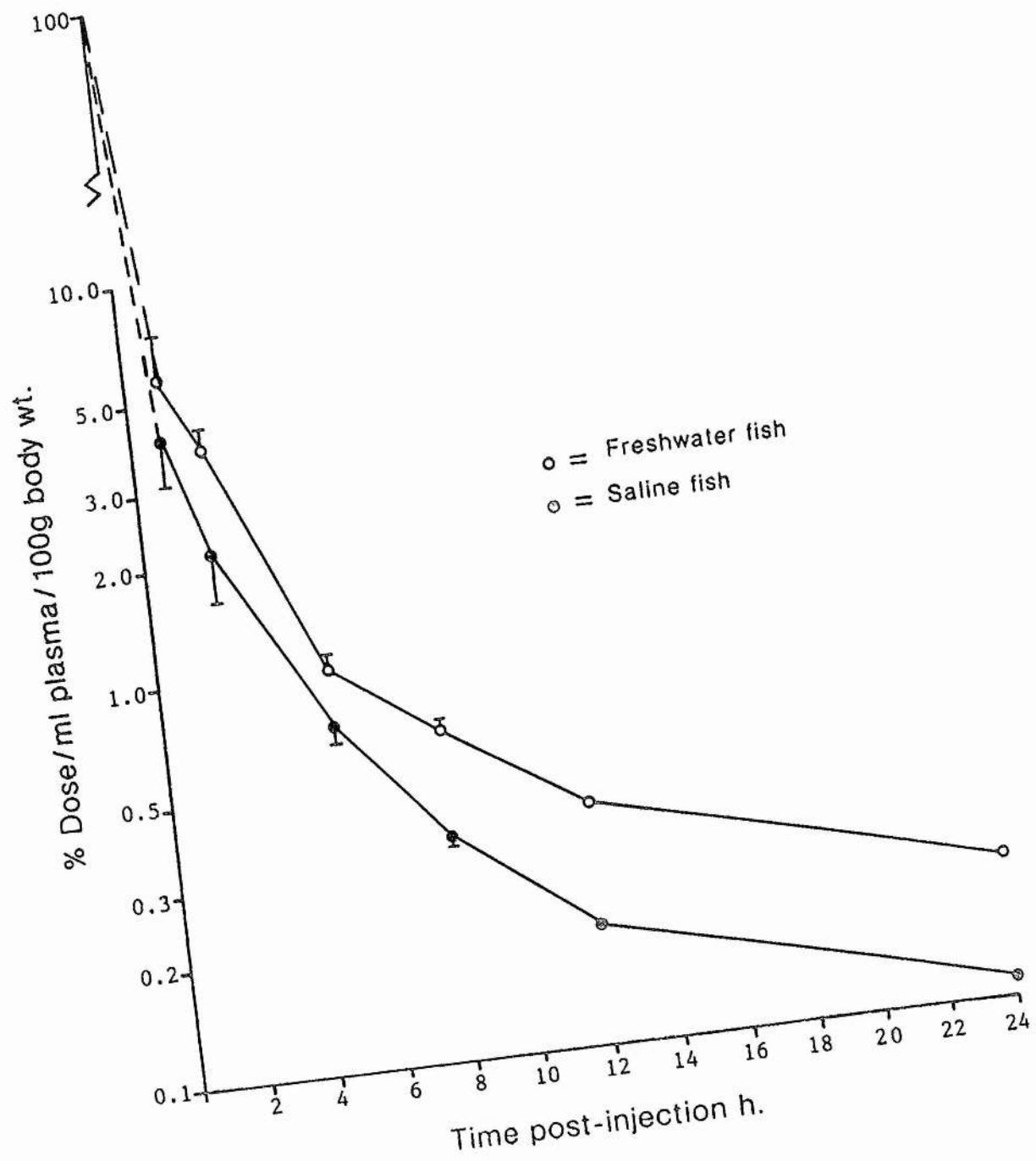
4.4.4 GLUCOSE DETERMINATIONS

Glucose was determined by the highly specific hexokinase [HK] method and based on a Sigma Diagnostics kit (115-5).

FIGURE 4:1

Typical Experiment to Determine the
Clearance Rate of Radioactivity from
Plasma After a Single Injection of
4 mCi ^3H Cortisol

A single injection of ^3H cortisol was made at
time 0 and blood samples were taken from
individual fish (groups of 3) at each time
point 1, 2, 5, 8, 13 & 24 hours after time 0



4.4.5 LACTIC ACID DETERMINATION

Lactic acid was determined by an enzymatic procedure based on the reduction of NAD to NADH by lactate dehydrogenase as determined by the formation of chromagens with an absorption maximum at 540 nm. The procedure was based on a Sigma diagnostic kit procedure No. 826-UV

4.4.6 FREE FATTY ACIDS DETERMINATION

Free fatty acids were measured by an enzymatic procedure based on the technique used in Boehringer Mannheim kit no. 1082914.

Plasma analysis and statistical analysis was performed as described in sections 3.3.4 and 3.3.5 in chapter 3.

4.5 RESULTS

4.5.1 GROWTH RATE EXPERIMENT POPULATIONS

Table 4:1 shows the plasma cortisol and plasma glucose concentrations and cortisol MCRs for the medium population group during the growth cycle.

As stated in chapter 3, there were no significant differences ($p > 0.2$, $n = 10$) in plasma cortisol concentrations between fresh and salt water groups at any time during the growth cycle (table 4:1). It is however interesting that the MCR of cortisol was always greater in saline adapted fish compared to freshwater fish (table 4:1).

Plasma glucose concentrations increased in both freshwater and saline water eels during the growth rate experiment ($p < 0.0001$) although there was no significant difference between the two groups for a particular time point ($p > 0.3$, $n = 10$) (table 4:1) except on day 39 where the plasma glucose concentration was significantly higher in the saline water fish ($p = 0.0225$). This overall increase in plasma glucose is somewhat surprising and perhaps reflects a change in sensitivity to the endocrines controlling glucose metabolism.

TABLE 4:1

Plasma Cortisol, Metabolic Clearance
Rate of Cortisol and Plasma Glucose
Concentration During the Growth
of Fish in Either Fresh Water or
Saline Water (12 ppt)

Plasma cortisol and plasma glucose values are means \pm SEM for 10 fish. Cortisol MCR values are derived from a single clearance experiment (see text). * indicates a significant difference between saline fish values ($p < 0.05$) and those in the fresh water group

Time (Days)	Plasma cortisol (ng/ml)		Plasma glucose (mmol/L)		Cortisol Metabolic clearance rate (ml/100g/h)	
	FW	SLW	FW	SLW	FW	SLW
39	17.9 ± 1.9	17.6 ± 3.1	1.27 ± 0.06	1.45 ± 0.04*	6.50	8.79
70	24.2 ± 3.1	17.8 ± 4.0	2.53 ± 0.60	2.94 ± 0.50	5.64	7.32
120	33.9 ± 5.0	40.4 ± 5.0	3.15 ± 0.08	3.27 ± 0.90	11.16	14.40
164	28.1 ± 3.2	36.7 ± 7.6	4.34 ± 0.45	4.44 ± 0.40	5.41	8.62
210	22.5 ± 2.4	19.8 ± 3.8	4.82 ± 0.22	4.47 ± 0.24	5.33	9.93
233	44.1 ± 5.4	48.0 ± 5.0	6.15 ± 0.20	6.35 ± 0.23	6.89	7.71

Plasma concentration of lactate and free fatty acids (table 4:2) were relatively constant throughout the experiment in both freshwater and saline water and there was no significant difference in the values of either parameter ($p > 0.3$ lactate, $p > 0.2$ PFFA) at any time during the experiment. PFFA concentration was however significantly decreased in the saline water group in comparison to the fresh water group at the end of the growth period ($p = 0.0006$).

TABLE 4:2

Plasma Lactate and PFFA Concentration
During Growth of Fish in Either Fresh
Water or Saline Water (12 ppt)

Values are means \pm SEM for 6 fish. * indicates
a significant difference between saline fish
values ($p < 0.05$) and those in the fresh water
group

Time (days)	Lactate mmol/L		PFFA (mmol/l)	
	FW	SLW	FW	SLW
39	2.73 ± 0.46	2.53 ± 0.40	0.42 ± 0.03	0.46 ± 0.8
70	3.27 ± 0.42	3.60 ± 0.31	0.54 ± 0.09	0.49 ± 0.13
120	3.01 ± 0.30	2.57 ± 0.51	0.39 ± 0.07	0.37 ± 0.06
164	3.4 ± 0.40	3.76 ± 0.31	0.63 ± 0.04	0.62 ± 0.1
210	3.20 ± 0.23	2.63 ± 0.49	0.34 ± 0.01	0.42 ± 0.07
233	3.28 ± 0.26	3.57 ± 0.14	0.38 ± 0.01	0.31 ± 0.01*

4.5.2 STOCKING DENSITY EXPERIMENT POPULATIONS

As reported in chapter 3 (section 3.4.2, table 3:4), at a stocking density of 130 kg/m^3 , plasma cortisol concentrations were significantly higher ($p < 0.01$) in the fresh water population than in the saline water population.

Plasma concentrations of glucose were higher in both groups at the high stocking density than in the lower stocking densities of the growth rate experiments (see tables 4:1 and 4:3). An increase in plasma glucose was observed in both groups as the experiment progressed, similar to that observed in the growth rate experiment. No significant difference ($p > 0.09$, $n = 12$) was observed between fresh and saline water groups (table 4:3) except on day 52 where there was a significant decrease in plasma glucose in the saline water fish ($p < 0.0001$).

Plasma concentrations of free fatty acids deviated little from those in the growth rate experiment fish (see tables 4:2 and 4:3). Although there was a significant reduction in the plasma concentration of PFFA in the saline water group at the start of the experiment ($p = 0.038$, $n = 12$), there was no such difference at the end ($p = 0.87$, $n = 12$) between fresh and saline water groups (table 4:3).

The MCRs of cortisol were slightly higher in saline water eels at ($12.6 \text{ ml/100 g/hour}$) than in freshwater eels ($10.8 \text{ ml/100 g/hour}$).

TABLE 4:3

Plasma Concentrations of Glucose
and Free Fatty Acids (PFFA) in Fish
Grown at an Initial Stocking Density
of 130 kg/m³ in Either Fresh Water
or Saline Water (12 ppt)

Results are means \pm SEM of at least 12 fish
in each group. * indicates a significant
difference between saline fish values
($p < 0.05$) and those in the fresh water
group

Days	Plasma glucose (mmol/L)		Plasma FFA (mmol/L)	
	FW	SLW	FW	SLW
0	4.80 ± 0.36	4.54 ± 0.42	0.38 ± 0.01	0.31 ± 0.03*
52	5.78 ± 0.24	3.08 ± 0.24*	—	—
137	8.09 ± 0.93	6.29 ± 0.44	0.37 ± 0.06	0.38 ± 0.06

4.6 DISCUSSION

The stress response enables animals (including fish) to withstand a potentially hostile environment. Perhaps the central element of the stress response is the change from an anabolic state (energy reserves stored) to a catabolic state (energy reserves broken down). This energy mobilisation is used by the fish to overcome or avoid the threatening situation. In aquaculture, fish are exposed to a variety of stresses of different intensity and duration (e.g. physical disturbance, increased stocking density, deterioration of water quality and disease treatments) and the opportunities to avoid or escape from such stresses are limited. Response to stress in such an environment does not confer benefits in terms of immediate survival but will, instead, lead to the development of a series of damaging side effects especially if the stress is prolonged (figure 3:6).

The stress response of cultured fish has been classified into three categories which are collectively known as the General Adaption Syndrome (GAS) as described in figure 3:6 in chapter 3:

1) **Primary (alarm reaction):** Neuroendocrine responses mediated by activation of the pituitary-interrenal axis (Donaldson, 1981) and stimulation of adrenergic function (chromaffin tissue) (Mazeud and Mazeud, 1981; Randal and Perry, 1992).

2) **Secondary (resistance):** Physiological consequences which may affect plasma electrolyte concentrations (Eddy, 1981) plasma glucose and intermediary metabolism (Leach and Taylor, 1982;) and immunosuppression (McLeay, 1973; Tripp *et al.*, 1987; Tort *et al.* 1995, 1996).

3) **Tertiary (exhaustion):** Consequences which include behavioural changes, increased susceptibility to disease and decreased growth rates (Wedemeyer and McLey, 1981; Barton *et al.*, 1987; Pickering, 1992).

During the growth rate experiment the large increase in growth rate of saline water fish (see section 3.4.1a, chapter 3) did not appear to be related to decreased plasma cortisol concentrations as both freshwater and saline water fish showed similar concentrations (table 4:1).

At a stocking density of 130 kg/m³, the significantly higher plasma cortisol concentrations ($p < 0.01$) (section 3.4.2, table 3:4), in the fresh water population compared to the saline water population suggests that the fresh water fish were relatively stressed. This was probably the result of a combination of crowding and osmoregulatory and ionoregulatory pressures. This increase in stress in the fresh water population could explain the reduction in growth within the group (figure 3:10 and table 3:3).

Plasma cortisol levels *per se* do not necessarily reflect the direct effect of a particular stress, rather plasma hormone concentrations represent the balance between interrenal gland secretion and clearance rates. The cortisol MCR was always greater in saline water adapted fish, even at the high stocking densities (130 kg/m³). This suggests that cortisol was removed or metabolised at a faster rate by the saline water fish. The consistent difference between clearance rates in fresh and saline water fish may reflect an increased incorporation of ³H cortisol into the liver, the main site of cortisol breakdown, or the bile, the major route for cortisol excretion. This would result in a reduction in the number of receptors which bind cortisol so reducing its possible detrimental effects.

Similarly, the increase in growth rate of the saline water fish appears to be unrelated to PFFA, lactate or glucose as the concentration of these did not vary between the salt and fresh water groups of fish. The use of these parameters as "stress markers" in eels will be discussed in chapter 5.

The understanding of the stress response in fish is far from complete particularly since the majority of research to date has concentrated on salmonid species. This study is the first detailed study of primary and secondary stress responses in the eel. It has a strong emphasis on commercial aquaculture and the results form the basis for identifying and reducing stress in order to maximise eel growth rates and increase profitability.

CHAPTER 5

Acute Stress

CHAPTER 5

ACUTE STRESS

5.1 ACUTE STRESS

Stress in aquaculture has been discussed in chapter 4 with particular reference to chronic stress. It is the purpose of this chapter to discuss acute stress which may frequently occur under commercial aquaculture conditions. While some activities such as handling, grading and netting fish are clearly acute stressors (Flos et al., 1990). In some cases, such as disease, the acute stressor if not treated can develop into a chronic stressor. Similarly, a frequently repeated acute stressor may eventually may become indistinguishable from a continuous chronic stressor. For example, regularly handled fish may exhibit a permanent stress response similar to chronic stress, (Sunyer et al., 1995), a condition termed daily acute stress.

Common acute stressors in aquaculture are:

5.1.1 HANDLING

Capture, grading for size, sex or maturity, stripping eggs and milt, weighing and lengthing, tagging and injecting are all part of modern day fish farming operations. Effects of handling have been shown to increase plasma cortisol concentration. These values return to normal levels depending on the duration of

handling, usually 13-24 hours later in juvenile chinook salmon *Oncorhynchus tshawytscha* (Strange and Schreck 1978) and six hours after handling juvenile chinook salmon for just 30 seconds (Barton *et al.*, 1986).

5.1.2 ANAESTHESIA

Anaesthetic use is common on the fish farm, especially when handling is a necessity (see above). Anaesthetics such as MS 222 (ethyl m-aminobenzoate methanesulfonate) and phenoxy-ethanol have been shown to cause increased levels of cortisol in the plasma of rainbow trout (Barton and Peters, 1982). As anaesthetization of the fish can be more stressful than short term handling, the fish farm manager has to assess whether the use of an anaesthetic is of benefit under certain circumstances. For example, in yearling chinook salmon, similar concentrations of plasma cortisol were found after handling from one tank to another as in fish which had been anaesthetized using MSS 222 (Strange and Schreck, 1978). It is therefore unwise to net the fish into a container of anaesthetic as the fish would be doubly stressed, the best procedure would be to add the anaesthetic to the water the fish are inhabiting. The volume of water may be reduced to economise on anaesthetic, however, care must be excercised to avoid such a reduction of water so as to induce stress due to crowding. A compromise between economy of anaesthetic and crowding stress must therefore be established.

5.1.3 CHEMICAL TREATMENTS

Various chemical treatments are used in fish farming to combat disease and also to restore the environmental parameters in recirculation systems. Formalin is added to control various ectoparasites and to control the ciliate blooms so common in recirculating systems. Malachite green is used to combat fungal infections, and Hyamin is commonly used against fin rot (see section 2.2.1d). Chemicals such as these cause varying degrees of stress, the cat fish *Clarias batrachus* is apparently so sensitive to formalin that treatment becomes impossible (Droogendijk, pers. comm.).

5.1.4 WATER QUALITY

Water quality is discussed in some detail with reference to recirculating systems in chapter 2 and with reference to chronic stress if reduced water quality is prolonged in chapter 4. Obviously any fall in water quality will produce some stress in fish but it should also be considered that adjusting certain parameters too rapidly, e.g. temperature and pH as discussed in chapter 2, can be equally stressful.

5.1.5 TEMPERATURE

This is discussed in some detail in chapter 2. Fish, depending on the species, have upper and lower lethal temperatures and the closer they come to these limits the more stressed they become. It is well known by fish farmers, that temperature fluctuations cause stress and the wider the range of these fluctuations, the greater the stress is. Steelhead trout when transferred from water at 10 to 20°C. were stressed but acclimated within 24 hours. A similar effect was observed in juvenile cut throat trout transferred from water at 13 to 26°C. (Strange *et al.*, 1977). Juvenile coho salmon showed no significant signs of stress when the daily temperature fluctuated between 9 to 15°C. but this was not the case when the daily temperature varied between 6.5 to 20°C. In the latter case this resulted in the stimulation of the HPI axis (Thomas *et al.*, 1986).

5.1.6 TRANSPORT

As technology improves, fish are being transported more frequently and over longer distances. The stressors involved in transportation include netting, handling, crowding in the transport vessel, variations in pO₂ and containing the fish in an unfamiliar environment. Transport stress is compounded when the fish are introduced to a different environment at the destination. This is a particular problem when fish are moved from fresh to salt water, such as in smolt transfers, where the

immune system is suppressed by increased levels of plasma cortisol. Several methods are commonly used to reduce the stress of transportation. These methods include making the water in the transport tank of equal ionic concentration to that of the extracellular fluid and administering a small amount of anaesthetic to the water. It is considered by some farmers that lowering the temperature of the transport tank will reduce the metabolic rate of the fish and hence subdue them. It must be remembered however, that the temperature shock exerted by such practice can also stress and so this technique may only be of net benefit if the transport tanks are not optimally oxygenated.

5.1.7 DISEASE

Disease is acutely stressful to fish and if not treated it becomes chronic. Disease is therefore referred to as chronic in chapter 4. Aspects of the stressful effects of disease have also been discussed in chapter 2.

5.2 CATECHOLAMINES

The primary effects of stress include increased cortisol and catecholamine (CA) secretion. However an increase in CA is rapid and normally regarded as a first indication of acute stress. The role of CAs in acute stress will now be discussed.

5.2.1 ADRENOCORTICAL MEDULLARY HOMOLOGUE

The adrenocortical medullary homologue is responsible for the secretion of the CAs. The most important CAs are: adrenaline (epinephrine), the closely related noradrenaline (norepinephrine) and dopamine. In fish, CAs are synthesised and stored in the chromaffin tissue (so called as it stains with chromates). Chromaffin cells are scattered throughout the body, their specific location depending on the species and have no close association with the interrenal tissue (Oguri, 1960). The chromaffin cells are located in cyclostomes (hagfish and lampreys) in the walls of the major cardinal veins, the sinus venosus and especially the muscle fibres of the heart (Augustinsson *et al.*, 1956, Bloom *et al.*, 1961); in the dipnoans (lung fish) the left caudal vein, inter costal arteries and atrium (Abrahamsson *et al.*, 1979). In teleosts there are small islets in the walls of the posterior cardinal vein containing the chromaffin tissue which are also scattered throughout the anterior head kidney (Nilsson 1983; Randall and Perry 1992) and

this is the case in the American eel *Anguilla rostrata* (Epple *et al.*, 1989). The elasmobranch fish show a greater degree of organisation by displaying small aggregates of chromaffin tissue lying linearly against the kidney. Termed supra renal bodies the anterior pair of these aggregates (axillary bodies) are well developed in some species (Gannon *et al.*, 1972)

5.2.2 STRUCTURE OF ADRENALINE AND NORADRENALINE

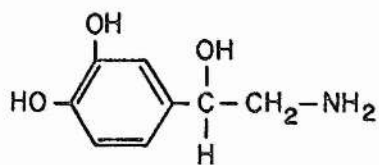
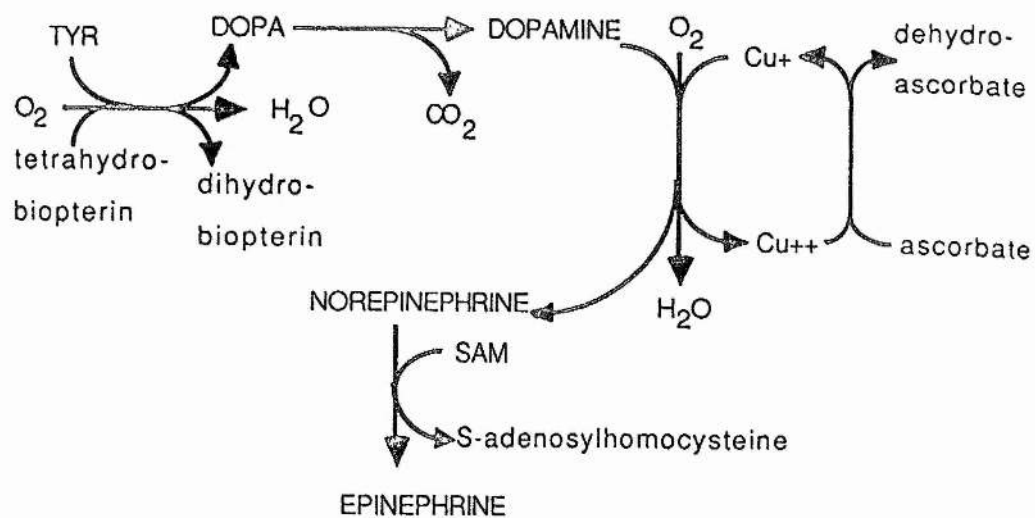
The structure of adrenaline is amino-hydroxyphenyl-propionic acid. Noradrenaline is identical but lacks a methyl group (figure 5:1). Synthesis of noradrenaline is from tyrosine which is hydroxylated into dopa, deaminated to dopamine and hydroxylated to noradrenaline. Noradrenaline may then be methylated to adrenalin (Blaschko, 1939). Excretion is exclusively via the urine (normally within 5 days) for both adrenaline and noradrenaline (Mazeaud and Mazeaud, 1981).

5.2.3 RELEASE AND EFFECTS OF CATECHOLAMINES

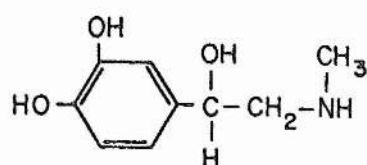
The release of CAS is under the control of the sympathetic nervous system (Randall and Perry, 1992), the chromaffin tissues being innervated by preganglionic cholinergic fibres (Nilsson, 1976). Electrical stimulation of these fibres causes a release of CAS in Atlantic cod *Gadus morhua* (Nilsson *et al.*, 1976).

FIGURE 5:1

Structure of D-Epinephrine (Adrenaline)
and D-Norepinephrine (Noradrenaline)



D - NOREPINEPHRINE



D - EPINEPHRINE

The response to electrical stimulation is very rapid, especially when compared with the release of cortisol following the activation of the HPI axis. This combination of fast CA release and slow cortisol release is the reason for the initial anabolic use of the fish's energy reserves, under the control of CAs, and the subsequent use of the catabolic pathways under the control of cortisol. In the latter case, proteins and lipids are mobilised to supply the fish's energy requirements (Pickering, 1992). Blood chemistry may also have an influence on the release of CAs such as oxygen depletion (Perry *et al.* 1991), increased $[K^+]$ in elasmobranchs (Opdyke *et al.*, 1983) and hagfish *Myxine glutinosa* (Perry *et al.*, 1993). Perry *et al.* (1993) induced the release of CAs in hagfish by injecting pituitary extract from the Atlantic cod.

Resting levels of adrenaline and noradrenaline are considered to be less than 10 nM and dopamine even less. Stress levels of CAs can be as much as 300 nM. (Gamperl *et al.*, 1994)

The effects of CAs are to stimulate respiratory, metabolic and circulatory systems which increase the delivery of oxygen to tissues with a high oxygen demand. CAs increase blood flow through the gill and decrease the blood flow in the central sinus compartment (Steen and Krusysse, 1964., Girard and Payan, 1976). In fresh water adapted perfused trout heads CAs also stimulate active ion transport mechanisms for Na^+ (Payan *et al.*, 1975) and Cl^- (Perry *et al.*, 1984). However, the opposite effect was observed *in vivo*. CAs have been shown to stimulate branchial

permeability to water and organic substances such as urea (Isaia, 1979). Other physiological adjustments induced by CAs include; the stimulation of the release of erythrocytes (RBCs) by the spleen into the blood thereby increasing the oxygen carrying capacity (Nilson and Grove, 1974); the regulation of the erythrocyte pH which preserves the oxygen carrying capacity of the blood (Nikinma *et al.*, 1984); increasing the breathing rate (Randall and Taylor, 1991); increasing the functional gill surface area and epithelial permeability to oxygen, thus increasing oxygen uptake (Perry *et al.*, 1985) and increasing the net H^+ equivalent flux allowing the blood pH, and hence oxygen carrying capacity, to return to normal (Tang *et al.*, 1988). Vasodilation is reported as a result of administering adrenaline to perfused gill preparations (Rankin and Maetz, 1971) which may be preceded by transient vasodilation (Wood, 1975). Also the hypothalamic pituitary-interrenal (HPI) axis is affected which causes the release of cortisol (Henderson and Garland, 1980). The effects of cortisol in the stress response are discussed in section 4.3.1 of chapter 4. A consequence of some of these respiratory and vascular adjustments is a net loss of ions in fresh water fish and gain in sea water (Pickering, 1992). In a farm situation, stress resulting in CA release will lead to an increase in oxygen consumption. For example, the oxygen consumption on a commercial trout farm was still elevated 24 hours after grading (Smart, 1981). CAs also mobilise stores of energy by stimulating hepatic glycogenesis thus maintaining blood glycogen levels (Mommensen *et al.*, 1988).

During acute stress e.g. handling, hypoxia, confinement or shock, CAs are released by the chromaffin tissues (Randall and Perry, 1992) within minutes (Mazeaud, 1981). Despite small levels of dopamine being discovered in the plasma of elasmobranchs (Butler *et al.*, 1978) and teleosts (Ristori *et al.*, 1979) it appears that, as a result of stress, the concentration of dopamine changes very little in dogfish (Butler *et al.*, 1978) and not at all in teleosts (Ristori *et al.*, 1979). The time taken for CAs to be released varies according to species. Cyclostomes (sea lampreys, *Lampréta fluviatilis*) release CAs 3 minutes after being bled during struggling and anoxia (Mazeud, 1969). Experiments on teleosts using carp, *Cyprinus carpio*, forced to swim on a hook and line or held under anoxic conditions. CA release occurred 15-30 minutes after the beginning of the experiment (Mazeaud, 1964). In rainbow trout (*Oncorhyncus mykiss*) a physical disturbance resulted in the release of adrenaline after 2 minutes and noradrenaline after 10 minutes (Nakano and Tomlinson, 1967). The function of CAs is to ameliorate the physiological effects of stress (Perry and Wood, 1989; Nikinma, 1992) through the mechanisms discussed above. The most important mechanisms in this respect are enhanced oxygen branchial transfer and transport by the blood.

5.3 MATERIALS AND METHODS

The aim of these experiments was to assess the acute stress effects of practical procedures which are performed on a regular basis in commercial aquaculture systems. These short term experiments were therefore designed to investigate the effects of two practical aspects of commercial eel aquaculture.

- 1) Grading eels through grading bars
- 2) Netting eels and removing them from tanks. This practice is performed on a routine basis for sample weighing, bath treatments, vaccination, cleaning tanks, moving to other tanks etc.

5.3.1 GRADING EXPERIMENT

Grading is a normal requirement in eel aquaculture as it is necessary to separate large fish from small ones in order to maximise growth. However, the stress involved may have detrimental effects including appetite loss. To assess grading stress, groups of 30 fish were taken from either the freshwater system (average weight 81.1 g.) or the saline water system (average weight 81.3 g.) and graded through bars spaced 13 mm apart (C&H Plastics). Blood samples were taken from 6 individuals at 0, 5, 20, 60 and 90 minutes post-grading. The fish were graded into an isolated tank at the start of the experiment. Samples were taken rapidly to minimise any further changes in blood

chemistry. This was achieved by adopting the following protocol: When a fish was required for blood sampling, a net was placed gently underneath the fish so as not to alarm it, the fish was then rapidly removed and decapitated to obtain the sample which was collected in a heparinized tube. The procedure was completed in 10-30 seconds and any samples taking longer were discarded. Plasma osmolality, haematocrits and plasma concentrations of chloride, cortisol, glucose, free fatty acids and lactate were determined as described above (sections 3.3 and 4.4).

5.3.2 NETTING EXPERIMENT

A frequent occurrence in aquaculture is the transfer of fish between different tanks as part of normal farm management. The effects of this procedure were determined by netting groups of 30 eels (90-130 g) from either a fresh water or saline water stock tank and then transferring them to an isolation tank. Blood samples were rapidly taken, as described above, from 6 individual fish in 5 groups at 0, 5, 20, 60 and 90 mins after transferral to the isolation tank. Plasma osmolality, haematocrits and concentrations of chloride, cortisol, glucose, plasma free fatty acids and lactate were determined as described in section 3.3 and 4.4 in chapters 3 and 4.

5.3.3 CATECHOLAMINE DETERMINATIONS

Plasma catecholamines were kindly measured by Mr. Simon MacKenzie during an ERASMUS exchange visit to the Universita Autonoma di Barcelona, Spain. Briefly, catecholamines were identified by HPLC analysis with electrochemical detection following the technique of Woodward (1982). Blood was collected into EGTA/glutathione centrifuge tubes and plasma separated and stored at -70°C . before use. Plasma was extracted using Al_2O_3 extraction techniques and chromatographed on an MN-HPLC column (Nucleosil 5 SA) with subsequent electrochemical detection.

5.3.4 STATISTICAL ANALYSIS

Statistical analysis was performed as described in section 3.3.5 in chapter 3.

5.4 RESULTS

5.4.1 GRADING EXPERIMENT

The experimental data from the acute grading stress experiments are summarised in tables 5:1-5:4 and figure 5:2.

Grading produced significant increase in plasma osmolality and chloride concentrations ($p < 0.05$) in both fresh and saline water groups of fish within 20 min after the grading stress (table 5:1) chloride in saline water fish increased after 5 mins. This may not be apparent in fresh water fish because of the larger S.Es of the samples. The increase was maintained for over 60 mins ($p < 0.05$) in both groups, these parameters (with the exception of chloride in fresh water fish) returned to pre-grading concentrations within 90 min.

Plasma glucose and cortisol concentrations following grading stress are summarised in table 5:2. The cortisol concentrations are also represented graphically in figure 5:2. In both groups plasma cortisol concentrations were significantly elevated 20 mins and 60 mins post-grading ($p < 0.05$) and in the freshwater fish, cortisol remained elevated at 90 mins ($p < 0.05$). The increase in cortisol was associated with a transient increase in plasma glucose concentrations which were significantly elevated after 20 and 60 mins in the fresh water adapted group and in the saline adapted group.

FIGURE 5:2

Acute Grading: Plasma Cortisol
Concentration in Either Fresh Water
or Saline Water (12 ppt)

Results are means \pm SEM of six fish in each group

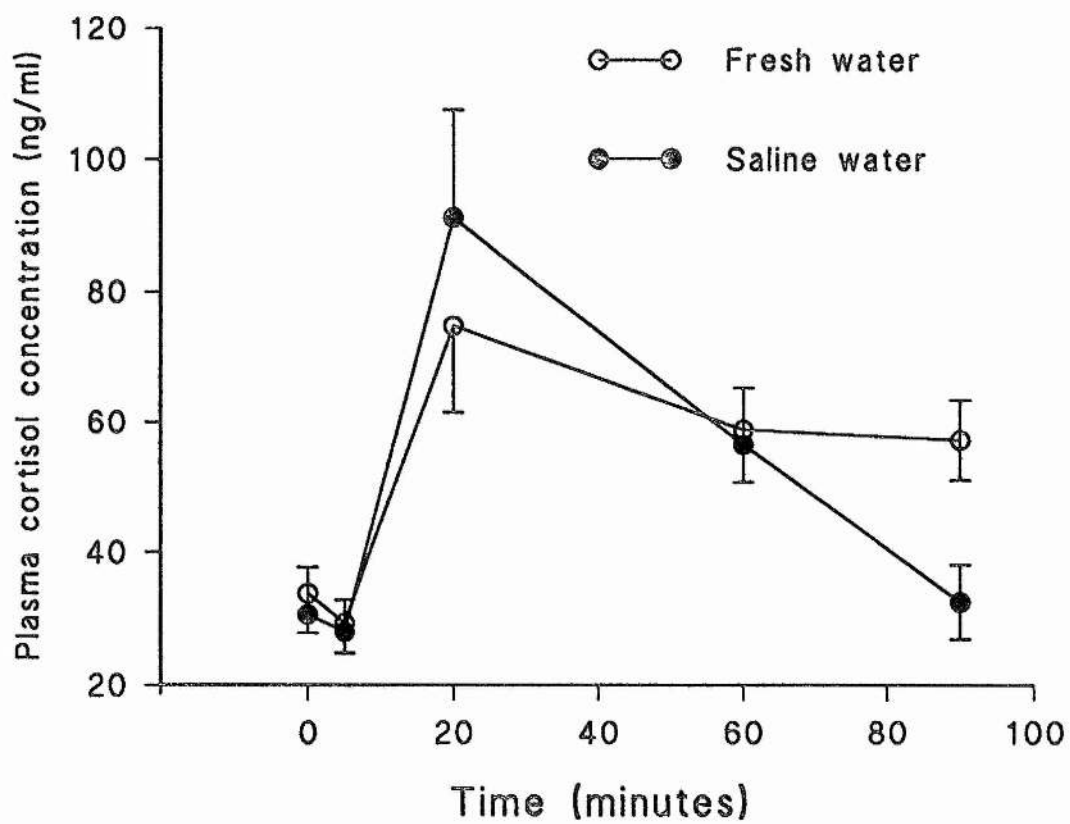


TABLE 5:1

Acute Grading: Plasma Osmolality
and Chloride Concentration in Either
Fresh Water or Saline Water (12 ppt)
Adapted Fish

Results are means \pm SEM of 6 fish in each group.

* indicates significant difference from samples
at time 0 ($p < 0.05$)

TIME					
Freshwater 0 ‰	0 mins	5 mins	20 mins	60 mins	90 mins
Osmolality mOsmol/kg	299.2 ± 3.0	299.3 ± 5.3	315.0 ± 2.4	312.6 ± 3.0*	304.5 ± 9.5
Chloride mmol/L	100.7 ± 3.1	103.7 ± 2.9	116.0 ± 3.1*	112.7 ± 3.7*	111.6 ± 4.3
Saline Water 12 ‰	0 mins	5 mins	20 mins	60 mins	90 mins
Osmolality mOsmol/kg	309.8 ± 2.2	316.0 ± 2.4	328.8 ± 3.4*	327.8 ± 6.8*	312.4 ± 5.2
Chloride mmol/L	102.2 ± 2.5	125.7 ± 1.8*	130.2 ± 2.3*	128.7 ± 2.3*	122.3 ± 3.4*

TABLE 5:2

Acute Grading: Plasma Glucose and
Cortisol Concentration in Either
Fresh Water or Saline Water (12 ppt)
Adapted Fish

Results are means \pm SEM of 6 fish in each group.

* indicates significant difference from samples
at time 0 ($p < 0.05$)

TIME						
Freshwater 0 ‰	0 mins	5 mins	20 mins	60 mins	90 mins	
Glucose mmol/l	6.08 ± 0.38	6.01 ± 0.5	6.88 ± 0.14*	7.5 ± 0.3*	6.09 ± 0.2	
Cortisol ng/ml	33.8 ± 6.0	29.3 ± 4.5	74.7 ± 13.2*	58.8 ± 8.1*	57.2 ± 6.2*	
Saline Water 12 ‰	0 mins	5 mins	20 mins	60 mins	90 mins	
Glucose mmol/l	6.3 ± 0.25	6.7 ± 0.2	7.4 ± 0.2*	7.6 ± 0.3*	5.6 ± 0.5	
Cortisol ng/ml	30.6 ± 7.1	28.0 ± 4.8	91.2 ± 16.2*	56.5 ± 8.8*	32.5 ± 5.6	

In both groups plasma glucose concentrations returned to pre-grading values after 60 mins.

There were no significant differences in plasma free fatty acids in fresh water fish at any time after grading (table 5:3). In saline water fish, a grading stress-induced increase was observed after 60 mins only ($p = 0.01$). Plasma lactate concentrations increased slowly after grading and were significantly elevated ($p < 0.03$) after 20 min in fresh water fish and after 90 minutes ($p = 0.01$) in saline water fish. There was, however, a marginal increase in plasma lactate concentrations ($p > 0.08$) in the saline water fish between 20 and 90 minutes.

Plasma haematocrits were very variable (30.8 % to 47.4 % in fresh water fish and 30.4 % to 46.7 % in saline water fish) and there were no apparent changes during the acute grading experiment in either fresh water or saline water. why?

Plasma catecholamine concentrations are shown in table 5:4. Levels of plasma CAs typically show considerable variations in fish. This is also found to be the case in this study. It is apparent that adrenaline concentrations are elevated almost immediately within 1-3 min in both fresh water fish ($p = 0.016$) and saline water fish ($p = 0.0037$). Rise in noradrenaline concentrations were not significant in the fresh water fish ($p = 0.29$) and saline water fish ($p = 0.18$), these results possibly give a poor indication of noradrenaline due to such large standard errors.

TABLE 5:3

Acute Grading: Plasma Lactate and
PFFA Concentration in Either
Fresh Water or Saline Water (12 ppt)
Adapted Fish

Results are means \pm SEM of 6 fish in each group.

* indicates significant difference from samples
at time 0 ($p < 0.05$)

TIME					
Fresh Water 0 ‰	0 mins	5 mins	20 mins	60 mins	90 mins
Lactate mmol/L	3.30 ± 0.13	3.69 ± 0.23	4.42 ± 0.25*	7.12 ± 1.38*	4.65 ± 0.40*
PFFA mmol/L	0.39 ± 0.04	0.40 ± 0.03	0.41 ± 0.02	0.40 ± 0.06	0.34 ± 0.03
Saline Water 12 ‰	0 mins	5 mins	20 mins	60 mins	90 mins
Lactate mmol/L	3.32 ± 0.14	3.20 ± 0.25	3.95 ± 0.31	4.01 ± 0.32	3.86 ± 0.10*
PFFA mmol/L	0.26 ± 0.02	0.21 ± 0.02	0.26 ± 0.01	0.33 ± 0.01*	0.29 ± 0.02

TABLE 5:4

Acute Grading: Plasma Catecholamine
Concentration in Either Fresh Water
or Saline Water (12 ppt) Adapted Fish

Results are means \pm SEM of 6 fish in each group.

* indicates significant difference from samples
at time 0 ($p < 0.05$)

Time	Freshwater 0 ‰		Saline water 12 ‰	
	Noradrenaline nmol/l	Adrenaline nmol/l	Noradrenaline nmol/l	Adrenaline nmol/l
Resting Values	24.8 ± 5.5	20.3 ± 4.5	23.4 ± 3.3	27.9 ± 4.0
Immediate post stress 1-3 minutes	37.3 ± 9.8	53.6 ± 10.6*	39.4 ± 10.7	52.6 ± 5.2*
5 minutes	30.1 ± 5.5	33.1 ± 8.2	30.9 ± 9.7	47.0 ± 11.6
20 minutes	38.0 ± 13.4	21.2 ± 4.9	18.8 ± 2.9	19.6 ± 3.3
60 minutes	25.8 ± 2.3	26.63 ± 2.3	17.2 ± 2.5	18.87 ± 2.4

It is also clear that catecholamines have returned to resting values within 20 mins after acute grading stress.

5.4.2 NETTING EXPERIMENT

The experimental data for the acute stress of net transfer are summarised in tables 5:5-5:8 and figure 5:3.

Plasma osmolality was raised transiently 20 mins after transfer in both freshwater and saline water fish (table 5:5). The significance of this transient rise in osmolality was greater in fresh water fish ($p = 0.02$) than in saline water fish ($p = 0.07$). There was no significant rise in plasma chloride concentrations ($p = 0.29$) in the fresh water fish and a slight rise ($p = 0.04$) in the saline water fish (table 5:5).

Plasma cortisol concentrations (table 5:6 and figure 5:3) were significantly increased after 5 mins in both groups ($p = 0.028$ fresh water, $p = 0.034$ saline water) and in freshwater fish remained elevated throughout the experimental period ($p = 0.018$ at 90 mins) whereas in the saline water fish, plasma cortisol returned to pre-stress levels after 90 mins ($p = 0.88$). In fresh water fish plasma glucose concentrations were increased within 20 mins of netting stress ($p < 0.0001$) and returned to control levels within 90 mins ($p = 0.018$) (table 5:6).

FIGURE 5:3

Acute Netting: Plasma Cortisol
Concentration in Either Fresh Water
or Saline Water (12 ppt)

Results are means \pm SEM of six fish in each group

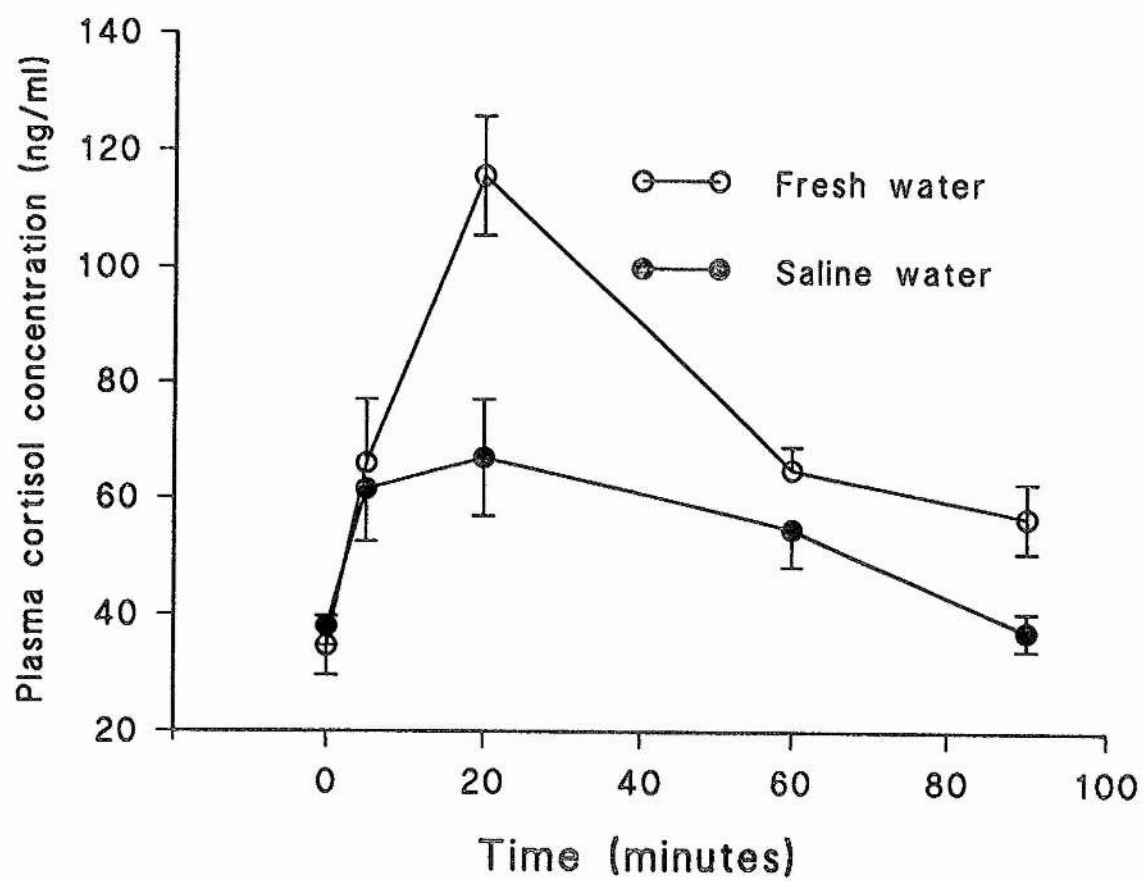


TABLE 5:5

Acute Netting: Plasma Osmolality
and Chloride Concentration in Either
Fresh Water or Saline Water (12 ppt)
Adapted Fish

Results are means \pm SEM of 6 fish in each group.

* indicates significant difference from samples
at time 0 ($p < 0.05$)

TIME					
Freshwater 0 ‰	0 mins	5 mins	20 mins	60 mins	90 mins
Osmolality mOsmol/kg	295.0 ± 3.8	298.0 ± 2.9	310.4 ± 4.1*	304.7 ± 3.8	294.0 ± 3.5
Chloride mmol/L	95.0 ± 3.8	96.6 ± 4.4	95.0 ± 3.5	101.7 ± 4.6	91.0 ± 7.4
Saline Water 12 ‰	0 mins	5 mins	20 mins	60 mins	90 mins
Osmolality mOsmol/kg	305.3 ± 5.4	309.0 ± 3.6	316.3 ± 0.9	303.6 ± 2.4	300.0 ± 4.8
Chloride mmol/L	115 ± 3.2	112.3 ± 3.6	122.3 ± 3.6	126.3 ± 3.6*	112.3 ± 4.2

TABLE 5:6

Acute Netting: Plasma Glucose and
Cortisol Concentration in Either
Fresh Water or Saline Water (12 ppt)
Adapted Fish

Results are means \pm SEM of 6 fish in each group
* indicates significant difference from samples
at time 0 ($p < 0.05$)

	TIME				
	0 mins	5 mins	20 mins	60 mins	90 mins
Freshwater 0 ‰					
Glucose mmol/L	4.36 ± 0.62	11.7 ± 3.91	16.29 ± 0.46*	5.04 ± 0.10*	5.48 ± 0.64
Cortisol ng/ml	34.7 ± 5.0	66.0 ± 11.1*	115.7 ± 10.1*	65.0 ± 4.1*	56.8 ± 6.0*
Saline Water 12 ‰					
Glucose mmol/L	14.18 ± 0.60	5.88 ± 0.56*	6.82 ± 0.51*	5.14 ± 0.77*	5.56 ± 0.54*
Cortisol ng/ml	38.0 ± 3.21	61.5 ± 9.0*	67.0 ± 10.1*	54.7 ± 6.4*	37.3 ± 3.2

However in saline water fish, plasma glucose concentrations were significantly reduced within 5 mins of netting stress ($p = 0.0001$) and this stress-induced fall in plasma glucose persisted for over 90 mins ($p = 0.0001$) (table 5:6). The glucose concentrations were, however, higher in the saline water group compared to the fresh water group ($p < 0.0001$) at the commencement of the experiment.

There were no apparent changes in plasma lactate or free fatty acids in either freshwater or saline water groups ($p > 0.1$) (table 5:7).

Plasma haematocrits showed no change following netting stress in either fresh or saline water fish.

Plasma catecholamine concentrations are shown in table 5:8. Within the first 5 minutes plasma catecholamine concentrations were increased but unfortunately there was insufficient volume of plasma available to obtain a complete set of data in this experiment.

TABLE 5:7

Acute Netting: Plasma Lactate and
PFFA Concentration in Either Fresh
Water or Saline Water (12 ppt)
Adapted Fish

Results are means \pm SEM of 6 fish in each group

	TIME				
	0 mins	5 mins	20 mins	60 mins	90 mins
Freshwater 0 ‰					
Lactate mmol/L	2.50 ± 0.30	1.62 ± 0.39	1.51 ± 0.21	1.82 ± 0.24	1.59 ± 0.18
PFFA mmol/L	0.29 ± 0.03	0.34 ± 0.04	0.22 ± 0.05	0.27 ± 0.04	0.31 ± 0.05
Saline Water 12 ‰					
Lactate mmol/L	1.42 ± 0.15	1.25 ± 0.06	1.16 ± 0.09	1.47 ± 0.08	1.78 ± 0.31
PFFA mmol/L	0.35 ± 0.03	0.36 ± 0.04	0.28 ± 0.07	0.30 ± 0.02	0.33 ± 0.01

TABLE 5:8

Acute Netting: Plasma Catecholamine
Concentration in Either Fresh Water
or Saline Water (12 ppt) Adapted Fish

Results are means \pm SEM of 6 fish in each group.

* indicates significant difference from samples
at time 0 ($p < 0.05$)

Time	Freshwater 0 ‰		Saline water 12 ‰	
	Noradrenaline nmol/l	Adrenaline nmol/l	Noradrenaline nmol/l	Adrenaline nmol/l
Resting Values	20.8 ± 4.6	22.8 ± 4.2	26.7 ± 6.0	23.7 ± 4.3
Immediate post stress 1-3 minutes	53.0 ± 7.2*	67.3 ± 8.9*	71.25 ± 9.7*	69.9 ± 12.5*
5 minutes	58.3 ± 11.6*	83.25 ± 14.9*	85*	70.3 ± 14.7*
20 minutes	39.4 ± 7.8	42.0 ± 8.6	52.5 ± 6.1*	42.9 ± 9.5
60 minutes	35.3 ± 4.5*	—	38.8 ± 8.8	—

5.5 DISCUSSION

The significance of acute stress was first demonstrated in initial experiments in this study which showed that the frequent removal of fish for weighing at intervals of 7 days had a detrimental effect on appetite, food consumption and growth rates.

The effects of acute grading stress and net transfer were qualitatively similar. The duration of the stress exerted by both grading and netting was approximately 30-60 seconds. However, the severity of the stress, according to the parameters measured in this study, of acute grading was found to be much greater than net transfer. Physical disturbances such as handling and confinement have been shown to increase circulating cortisol levels in a variety of species (reviewed by Gamperl, Vijayan and Boutilier, 1994) including salmonids (Pickering and Pottinger, 1987) and pleuronectids (White and Fletcher, 1989). It is very difficult to make comparisons between studies on this subject because of the variation in experimental conditions and species. For example, 10 seconds of confinement of rainbow trout, or tank transfer of chinook salmon resulted in a 2 hour elevation of plasma cortisol (maximal concentrations 40 ng/ml and 100 ng/ml respectively). The extent of these stress-induced elevations in cortisol are similar to those obtained for European eel in the present study. However, much larger elevations of plasma cortisol concentrations were induced by a 9 minute confinement of Atlantic

salmon in sea water (maximal 160 ng/ml from an initial concentration of 16.3 ng/ml) for 24 hours (Waring *et al.*, 1992). One possible reason for the relatively long duration of this stress response is the temperature to which the salmon were acclimated (10-12°C.) the return to resting levels would be expected to be faster at high temperatures (Barton and Schreck, 1987). In the present study, both groups of fish were held at 23°C. The recovery of plasma cortisol to resting levels was faster in saline water fish. This perhaps reflects the elevated metabolic clearance rate of cortisol (see chapter 4) in saline fish compared to freshwater fish and suggests that the saline fish recover more rapidly from acute stress such as grading or handling.

Hyperglycaemia in response to physical disturbances such as handling has been observed in many species (Soivio and Oikari, 1976; Pickering *et al.*, 1982; Waring *et al.*, 1992). Again there is variation between studies and species but in general the hyperglycaemic response is thought to reflect the increased energetic demands imposed on fish at times of stress. In the present study, plasma glucose concentrations increased within 20 mins of applying stress but returned to pre-stress levels within 90 mins. The transient nature of this response would be expected for acute stress and indicates that rapid changes in plasma glucose, particularly if paralleled by an increase in plasma cortisol concentration, is a good indicator of acute stress in eels. From the data obtained in this study, there appeared to be very little change in PFFA concentrations in fresh and saline

water eels following either grading or netting. PFFA would appear therefore not to be a good indicator of stress in the eel. This is not the case for some other species; plasma free fatty acids (PFFA) have been reported to increase in response to acute stress in Atlantic salmon *Salmo salar* and flounder *Platichthys flesus* and in some cases may represent better indices of stress (Waring *et al.*, 1992).

The effects of physical disturbances on plasma lactate concentrations vary according to species. For example, severe exercise induces large elevations of plasma lactate in rainbow trout (Turner *et al.*, 1983a; Milligan and Wood, 1986) but no change in pleurnectiforms (Turner *et al.*, 1983b; Milligan and Wood, 1987). In the present study, lactate concentration did not increase after acute stress exerted by net transfer in either freshwater or saline water groups. After grading stress there was a slow increase in plasma lactate concentration over the first hour post-stress in freshwater fish but not in saline water fish.

Changes in plasma ion levels such as sodium, potassium, chloride and plasma osmolality following acute stress such as handling have been reported in a variety of teleost species (Eddy, 1981; Robertson *et al.*, 1987; Waring *et al.*, 1992). Whether these stress induced changes are due to a change in plasma water (Wood and McDonald, 1982; Waring *et al.*, 1992) or to effects on branchial ion flux (Pic, 1978) are still unclear. It seems that acute changes in plasma ion levels after handling stress may be related to increased catecholamine levels at least in salmon and

flounder (Pic *et al.*, 1974). However there is no direct evidence linking stress induced changes in catecholamines or any other hormone to effects on branchial ion flux or osmotic permeability. In the present study, following grading induced stress, catecholamines were elevated rapidly and changes in plasma concentrations of ions occurred within 60 mins in the fresh water fish and 5 mins within the saline water fish after this response. Elevations in cortisol occurred concurrently with changes in plasma osmolality and chloride concentrations which suggests that these effects were most likely catecholamine mediated.

The data obtained in the present study indicate that elevation in plasma catecholamines and cortisol concentrations are the most sensitive indicators of stress in eels. In more severe and prolonged stress, glucose plasma concentrations may also be elevated. The saline water fish appeared to recover from acute stress (as indicated by catecholamine and cortisol) more rapidly than fresh water fish. This could contribute to the increased growth rates observed for saline water eels and be economically significant in commercial situations which demand frequent grading and movement of fish within the farm.

CHAPTER 6

Discussion

CHAPTER 6

DISCUSSION

6.1 ECONOMIC ASSESSMENT

6.1.1 ECONOMIC ANALYSIS (MICRO-ECONOMIC LEVEL)

This study has generated results that are of considerable economic value to the commercial culture of eels in closed recirculation systems. In particular, for the on growing of eels:

1) Eels grown in 12 ppt saline water showed more rapid growth than in freshwater. Currently freshwater is the normal medium for such culture.

2) The results indicate that eels maintained in saline water show reduced stress levels at high stocking densities and recover more rapidly from acute stress. Eels grown commercially in saline water could therefore be grown at higher stocking densities than fresh water eels.

6.1.2 ON GROWING EELS

In order to indicate the potential economic benefit of utilising the results of this study in a commercial situation, a simple analysis of direct variable production costs has been produced (see financial analysis 6:1; 1 and 1(a)).

FINANCIAL ANALYSIS 6:1; 1 and 1(a)

Financial Projections of a
Commercial Eel Farming Operation
Outlining the Advantage of Growing
Eels at Salinities of 12 ppt

FINANCIAL ANALYSES ASSUMPTIONS

FRESH AND SALINE WATER CONDITIONS

1. The cost analyses are based on the cost of purchasing 200,000 10g fingerlings at current market prices.
2. Feed, oxygen and electricity costs are based on a given rate per Kg of produced eel and are based on current aquaculture production costs.
3. All fixed costs such as labour, rent and rates, financing and transport costs have deliberately been excluded. This is on the basis that they are not influenced by the production environment, and as such, are not relevant for purely comparative purposes.
4. The economic summary does not give a complete gross profit as certain costs have not been included for the reasons stated above.
5. All figures are in pounds sterling.

ANALYSIS 1

SALINE WATER

	1	2	3	4	5	6	7	8	9	10	11	12	MONTHS
Stock of Eels at start of month(Kg)	0	2,500	3,125	3,906	4,883	6,104	7,629	9,537	11,921	14,424	17,453	21,119	
Purchase of Eels (Kg)	2,000	0	0	0	0	0	0	0	0	0	0	0	
Growth in Kg	500	625	781	977	1,221	1,526	1,907	2,384	2,503	3,029	3,665	4,435	
Stock at end of month (Kg)	2,500	3,125	3,906	4,883	6,104	7,629	9,537	11,921	14,424	17,453	21,119	25,554	
EXPENDITURE	£	£	£	£	£	£	£	£	£	£	£	£	TOTAL
Purchases	40,000	0	0	0	0	0	0	0	0	0	0	0	40,000
Feed	618	772	965	1,206	1,508	1,884	2,356	2,944	3,092	3,741	4,527	5,477	29,089
Oxygen Costs	125	156	195	244	305	381	477	596	626	757	916	1,109	5,888
Electricity	90	112	141	176	220	275	343	429	451	545	660	798	4,240
Heating	500	500	500	500	500	500	500	500	500	500	500	500	6,000
Treatment Costs	400	400	400	400	400	400	400	400	400	400	400	400	4,800
TOTAL	41,732	1,941	2,201	2,526	2,932	3,441	4,076	4,870	5,068	5,943	7,003	8,284	90,017

VALUE OF STOCK @ £5.90 PER KG £ 14,750 £ 18,438 £ 23,047 £ 28,809 £ 36,011 £ 45,013 £ 56,267 £ 70,333 £ 85,104 £ 102,975 £ 124,600 £ 150,766 £ 150,766

FRESH WATER

	1	2	3	4	5	6	7	8	9	10	11	12	MONTHS
Stock of Eels at start of month(Kg)	0	2,400	2,880	3,456	4,147	4,977	5,972	7,166	8,600	10,234	12,178	14,492	
Purchase of Eels (Kg)	2,000	0	0	0	0	0	0	0	0	0	0	0	
Growth in Kg	400	480	576	691	829	995	1,194	1,433	1,634	1,944	2,314	2,753	
Stock at end of month (Kg)	2,400	2,880	3,456	4,147	4,977	5,972	7,166	8,600	10,234	12,178	14,492	17,245	
EXPENDITURE	£	£	£	£	£	£	£	£	£	£	£	£	TOTAL
Purchases	40,000	0	0	0	0	0	0	0	0	0	0	0	40,000
Feed	494	593	711	854	1,024	1,229	1,475	1,770	2,018	2,401	2,858	3,400	18,828
Oxygen Costs	100	120	144	173	207	249	299	358	408	486	578	688	3,811
Electricity	72	86	104	124	149	179	215	258	294	350	416	496	2,744
Heating	500	500	500	500	500	500	500	500	500	500	500	500	6,000
Treatment Costs	400	400	400	400	400	400	400	400	400	400	400	400	4,800
TOTAL	41,566	1,699	1,859	2,051	2,281	2,557	2,889	3,286	3,620	4,137	4,752	5,484	76,183

VALUE OF STOCK @ £5.90 PER KG £ 14,160 £ 16,992 £ 20,390 £ 24,468 £ 29,362 £ 35,235 £ 42,282 £ 50,738 £ 60,378 £ 71,850 £ 85,501 £ 101,747 £ 101,747

ANALYSIS 1(a)

	<u>SALINE</u> <u>WATER</u>	<u>FRESH</u> <u>WATER</u>
TOTAL WEIGHT AT START (KG)	2.000	2.000
TOTAL WEIGHT AT END OF 12 MONTHS (KG)	25.554	17.245
RELATIVE INCREASE IN BODY WEIGHT	12.78	8.62
SALES VALUE AT £5.90 PER KG	£ 150,766	£ 101,747
LESS: DIRECT PRODUCTION COSTS	£ 90,017	£ 76,183
TOTAL GROSS PROFIT	£ 60,749	£ 25,563
GROSS PROFIT MARGIN	40.3%	25.1%
ORIGINAL COST OF ELVERS	£ 40,000	£ 40,000
GROSS PROFIT	£ 60,749	£ 25,563
RETURN ON INITIAL OUTLAY	151.9%	63.9%

This is not intended to be a detailed cash flow of a fully commercial and operational eel farm and the analysis is based on a series of assumptions (see assumptions - financial analyses 1 and 1(a)). The general basis of the analysis is only to include direct variable costs to enable comparisons to be made between the two systems. It has deliberately excluded fixed costs (labour, rent, rates, etc.) which are not influenced by the change in culture conditions and therefore have no bearing on this comparison.

The analysis 1 and 1(a) shows that the 12 ppt saline water environment produces a greater total biomass for the same time period. After 12 months, from an initial stock of 2,000 kg, the stock in fresh water reached 17,245 kg while the stock in saline water reached 25,554 kg. This represents an increase in growth for this period of 154 % in saline water compared to fresh water. This converts into a higher sales value. The increased production costs (expenditure) in salt water compared to fresh water reflect the superior growth rate achieved and subsequent greater total fish biomass. Analysis 1(a) shows a comparison of Gross Profit Margins increasing from 25.1 % in freshwater to 40.3 % in saline conditions and a Return on Initial Outlay of 63.9 % (fresh water) and 151 % (saline water) (it should, however, be noted that fixed costs are not included, see Financial Analyses Assumptions 3 and 4). This would represent a significant improvement in economic performance for ongrowing eel production. The figures given in the analysis are based on existing commercial eel farms (Stahler GMBH, Germany) growing eels at stocking densities of less than

100 kg/m³. The results in chapter 3 show that it is possible to grow eels at higher stocking densities in saline water compared to fresh water, therefore a further commercial advantage is to be obtained by growing eels in saline water.

Further research on economic aspects is essential because recirculating aquaculture requires relatively large amounts of start up finance which usually involves outside investors such as investment banks or venture capital. It is necessary to demonstrate to these investors in the clearest possible way the economic viability of new projects in order to ensure that recirculating aquaculture continues to develop within the E.U.

6.1.3 ECONOMIC ANALYSIS (MACRO-ECONOMIC LEVEL)

Although a macro economic analysis is beyond the scope of this study, some pertinent points are discussed in this section.

1) Within the E.U., the total production of eels is some 40-45% from wild catches and 55-60% from aquaculture. However the wild catch is continually falling due to environmental deterioration and the fish are often of variable quality and consequently have a lower value. There is therefore a requirement for efficient cost effective aquaculture of the eel to meet the E.U. market demands.

Approximately 50-60% of total aquaculture production comes from traditional lagoon type culture and 30-40% from modern intensive (often recirculating) aquaculture systems. A small quantity of around 10% is produced from semi intensive ponds or raceway systems. Intensive recirculating systems represent the most rapidly developing sector of eel aquaculture and improvements in production systems are vital to maintain the production of high quality, high value products. The rewards for the further development of intensive recirculating aquaculture production of eels will be increased profitability and financial security.

The product of recirculating systems is constant in supply and quality. Eels cultured in this way appear to be consistently silver and of high fat content. Wild eels are seasonal in quality and quantity depending on the stage in their life cycle. The wild silver eel is only available in the as it migrates towards the sea in the Autumn and it is at this stage that the fat content is high. A high fat content is much coveted by the smokers as it helps cook the eel during the hot smoking process and also adds to the flavour. The eel market pays less for brown/yellow eels, the stage at which the eel lives most of its life in fresh water, because of its appearance and relatively low fat content. The constant supply of eels from recirculating systems enables the farm to regularly harvest the mature eels and predict the future production with a high degree of certainty.

2) One of the major benefits of closed recirculating aquaculture systems is that they produce little or no effluent. This is very

desirable from an environmental perspective but it is likely to be of increasing economic significance as new legislation at both national and E.U. levels demands a reduction in effluent and imposes a "polluter will pay" principle. Environmental problems, discussed in the first Chapter, have resulted in siting for new fish farming ventures, at least in the U.K., becoming increasingly more difficult. New environmental legislation will therefore severely limit the profitability and expansion of traditional aquaculture production or semi closed recirculating systems.

3) The adoption of closed recirculating systems allows aquaculture systems to be located away from traditional locations that are often coastal sites with fragile ecosystems. From an economic point of view, this means that aquaculture can now be developed in much wider areas of the E.U, including economically deprived areas that require economic redevelopment.

4) Mono culture has been prevalent in the Scottish fish farming industry and this has imposed severe restrictions on its profitability and expansion. The reason only salmonids are cultured is that they are easily bred and reared in the open sea, rivers and lochs of mainly the west coast of Scotland. Once control of the environment is gained, the choice of species is only limited biologically by the ease with which it can be reared and economically by the value of the fish at market. Fish successfully farmed using recirculating systems include many species of bass, bream, carp, eels, salmon smolts sturgeon,

tilapia, trout and turbot. This has lead to the development of markets which specialise in exotic products which may be of limited value elsewhere in the world due to over production but have a high value locally. Examples of this are tilapia culture in Switzerland and sturgeon culture in Germany and Denmark.

6.2 FUTURE WORK

6.2.1 FUTURE WORK TO REALISE THE COMMERCIAL VALUE OF THIS RESEARCH

This study has demonstrated several advantages of growing eels at salinities of 12 ppt. These results are potentially of highly significant economic value to eel aquaculture but this research should be extended. In particular a full economic analysis of benefits to eel aquaculture is required using a fully commercial system. There are no obvious reasons why the findings of this study should not be transferable to any closed recirculating system. Although it would be necessary to demonstrate the financial benefits of using 12 ppt saline water, this is unlikely to present a problem (see section 6.1.2).

6.2.2 FUTURE SCIENTIFIC RESEARCH INTO EEL CULTURE

Eel aquaculture within the E.U. would greatly benefit from research into a number of key areas:

1) Perhaps the most important and most challenging problem is to develop methods to allow reproduction of eels in captivity. In particular larval development of the eel requires investigation together with the development of appropriate diets required during the early stages of the life cycle. Should this research

prove successful, the benefits would be enormous as it would overcome the constraints of catching wild elvers and relieve the pressure on wild eel stocks.

2) Basic scientific research is required into the factors that determine sexual differentiation in eels. Eels have no definite sex as elvers and differentiate according to many environmental factors. Matsui (1952) noted that of 8,558 Japanese eels in crowded ponds, only 50 were female suggesting that crowding increased the proportion of males. Burnet (1969) proposed that the increased proportion of males in crowded, wild, populations is due to increased competition for food. This would lead to poor nutrition and an increase in the number of males which are smaller than females. Win et al. (1975) found an increased proportion of females in estuarine areas of North American rivers. This could be due to less migrating elvers inhabiting these areas and keeping population densities low, an abundance of food due to increased productivity in saline waters or the direct result of saline water. Generally, female eels grow much larger and faster than males (Shinha and Jones, 1967) and therefore there would be economic advantages in growing more female fish. Egusa and Hirose (1973) concluded that in pond culture, males grow as fast as females and salinity was found to have no effect on male female ratios (Shina and Jones 1966). However in intensive culture systems, the proportion of female fish is small. This may relate to the environmental parameters used in culture and to increased effects of stress. At present, the sex of eels may be manipulated using steroid treatments but

this is unacceptable in commercial situations. This area would clearly benefit from more work into sexual differentiation from the earliest stage of weaning to mature adult fish. It is possible that the techniques developed in this study could be applied to investigate the effects of environmental parameters (e.g. temperature, salinity and stress) on sexual differentiation. The manipulation of sex ratios using these techniques would be commercially acceptable.

6.3. PROBLEMS ENCOUNTERED IN ADOPTION OF RECYCLING SYSTEMS

The advantages of closed recirculation aquaculture systems are numerous and are detailed in section 1.3 of chapter 1. However, the adoption of such systems commercially level has been slow, possibly due to the high capital investment required. Other reasons have been suggested by Rosenthal (1993) who concluded that because this area of the industry is new and optimistically promised short pay-back periods, it attracted mostly business investors with no previous aquaculture experience. The results reflected this and new companies were ignorant of the managerial and technical aspects of running such a complex biological system. Poor design is also cited as a reason for early failures with water purification companies offering inadequate systems for sale. Biofilters were undersized in order to cut costs in capital investment and save space which was at a premium in purpose built insulated and waterproof membrane sealed industrial units. One of the commonest failings of these systems resulted from the assumption that the systems were stable and metabolites were produced at a uniform rate and oxygen demands were constant. In reality, oxygen demands increase after feeding with the production of metabolites lagging behind (Poxton and Allouse, 1987)

6.4 AN OVERVIEW OF TECHNIQUES ADOPTED IN RECIRCULATION SYSTEMS

6.4.1 BIOFILTRATION

The biofiltration system used in this study was very successful in maintaining low levels of ammonia and nitrite. The combination of fixed film and activated sludge processes allow close to a 100% recirculation of water with the only additional water being that used to compensate for evaporative loss. This was possible due to constant monitoring of water parameters/quality and carefully adjusting the feeding regimes of the fish to the capacity of the biofilters.

The majority of alternative commercial systems adopt some form of mechanical filtration to remove solid wastes. Techniques range from lamellar plate separators, swirl separators, microstrainers and traditional settlement tanks. Removal of solids is best achieved as close to the effluent of the fish holding tanks as possible to avoid solid wastes dissolving into the water. Although this means that the waste is not being 100% recycled, removing solids from the farm alleviates many treatment problems. Solid wastes, once separated, may be dried and sold as high quality plant fertilizers as is the practice in Holland, or they can be sprayed, in liquid form, on crops or pastures near to the farm as practised in Denmark. Preliminary work has been conducted on the feeding of aquatic worms on these solids to produce bait for anglers. These worms could also, potentially, be recycled

through the fish farm by feeding them to the fish (Hazon and Bentley, Pers. Comm.).

Biofilters vary in design but typical systems operating in Europe today consist of a combination of trickling down-flow filters, one wet (submerged) and one dry (open to the air) Dry biofilters also contribute to oxygenation of the system. The two filters are often installed into the recirculating water circuit in parallel to each other and act as backups should one filter fail or be required to be closed down for maintenance. Trickling filters are favoured as they are relatively inexpensive and have a unique zoning, separating heterotrophic and nitrifying bacteria, thus eliminating competition for substratum and oxygen. By having a reduced flow to a submerged gravel filter in parallel with the main filters there has been some success in anaerobic denitrification (reduction of NO_3^- into N_2 and O_2).

6.4.2 ACTIVATED SLUDGE

The activated sludge process is not currently favoured in aquaculture systems as the flock remains too young. This is perhaps due to the activated sludge process being better adapted to the high loading, low volume situation of domestic sewerage treatment plants for which it was originally developed. The activated sludge process is consequently poorly understood by the aquaculturist. This process performed well in this study, possibly because solids were treated and not removed and the

nutrient loading was higher, producing an effluent more similar to the one encountered in sewerage treatment plants. As more conventional systems remove the waste solids, the water has a lower nutrient loading. The developing recirculating aquaculture industry is therefore ignoring a process which has the potential to provide many benefits.

6.4.3 FLUIDISED BEDS

Fluidised beds are not favoured because of the additional energy required to force the influent water up through the substrate (usually sand). In addition, the high turbidity of the substrate is very stressful on the containers, often causing them to rupture.

6.4.4 BIODISCS

Biodiscs such as those successfully used in this study are not universally adopted because of the relatively high demands on capital costs, space and running costs.

6.4.5 ULTRA VIOLET TREATMENT

UVc (260 nm wavelength) units are commonly used on recirculating fish farms although they have generally been added to existing

systems after problems with water quality occur eg bacterial blooms. Using UVc units in this way treats the problem and not the cause as the bacterial bloom is usually the result of excess nutrients in the water due to insufficient biofiltration capacity. By properly sizing the biofilter, bacterial blooms can be eliminated and in general the UVc filter should not be required.

UVc units have been reported to significantly reduce fish diseases. Rainbow trout held in UVc irradiated water infected with *Myosoma cerebralis* (causative agent for whirling disease) for 4.5 months showed very little signs of the disease compared with the control (Hoffman, 1975). In addition, the fish in UVc irradiated water had average weights 1.8-2.6 times greater than the control fish. UVc water treatment in a through-flow hatchery reduced deaths from the visceral myosporidian *Ceratomyxa shasta* from 60 % to 2 % in a coho salmon, *Oncorhynchus kisutch* and from 20 % to 1 % in trials on rainbow trout (Sanders et al., 1972). Atlantic salmon *Salmo salar* were prevented from contracting furunculosis, caused by *Aeromonas salmonicida*, by UVc irradiating spring water introduced to the hatchery (Bullock and Stuckley, 1977). UVc has limitations in controlling disease outbreaks once established. For example UVc kills the free swimming stages of the gill parasite *Oodinium* or *Ichthyobodo* (costia) but the adult stages remain on the fish. In this case UVc is used to break the life cycle but for maximal effect it may require to be used in conjunction with ozone.

A UVc unit could be incorporated into an existing system as an additional safety measure for use during outbreaks of epizootic diseases especially during the summer months. It is particularly useful in the sterilisation of influent make-up water to avoid the introduction of pathogens such as *M. cerebralis*, *C. shasta* and *A. salmonicida* into the system. In a 100% recirculation system the requirement for UVc is therefore limited.

6.4.6 OZONATION

Ozone, O_3 , is produced by passing dry refrigerated air, (O_2), over two arcing electrodes and is a powerful oxidation agent often producing valuable oxygen as a by-product. Redox potential in the recirculating water is a measure of free ions in solution. A negative potential indicates an abundance of reduced (negatively charged) molecules and is considered to be an indication of impure water while a positive redox potential indicates pure water. The addition of organic matter will reduce the redox potential, due to an abundance of reduced molecules, even if oxygen levels are at saturation. The redox potential can be restored by the addition of an oxidising agent such as potassium permanganate, hydrogen peroxide or ozone. The redox potential of natural sea water at pH 8.0 is 350–400 mV (Breck, 1974) a level considered satisfactory for aquaculture. At 700 mV the water is considered sterile. Ozone, when added to recirculation systems, initiates an increase in BOD as it is responsible for breaking carbon double bonds of large molecules.

These molecules which were previously highly resistant to biodegradation and hence unavailable to the heterotrophic bacteria in the biofilter, after ozone treatment can be utilised by these bacteria and hence increase the BOD. Such compounds would normally concentrate themselves in the water, causing discolouration and can only be removed by water changes. The clarity of the water can therefore be significantly improved by use of ozone treatment. Antioxidants, which are common in fish foods, may build up and inhibit the biofilter if not removed by partial water changes or ozonation. Ozone oxidises and breaks down potentially toxic compounds and is also capable of oxidising ammonia and nitrite thus assisting the biofilter in their removal. A number of compounds which can be removed by ozone are listed in appendix 1.

The use of ozonation in new recirculation systems seems an attractive option. The major draw backs are that ozone itself is lethal to fish (and operators) and must be removed from the water before entering the fish holding tanks. In order to avoid stressing the fish, ozone concentrations should be kept below 2 $\mu\text{g/l}$. There is some evidence to suggest that ozone produces compounds which may be lethal to oyster larvae (DeManche et al., 1975). These compounds can only be removed by treatment with activated carbon (this treatment also removes ozone). Ozonation requires expensive equipment as does its removal before returning the water to the fish. Ozone addition is via contact chambers and removal by either passing the water through activated carbon, aeration of the water, vacuum degassing or by UVc treatment.

Dealing with the removal of ozone from large quantities of water is a problem. There is little opportunity to treat the water, as in a fish farm, a continuous flow must be maintained. Therefore monitoring ozone is necessary, expensive and not always reliable.

Pathogens such as viruses and bacteria are destroyed by protoplasmic oxidation using ozone (Fetner and Ingols, 1956) as are protozoans and to some extent rotifers (Giese and Christensen, 1954). The growth of juvenile eels, *Anguilla anguilla*, is significantly improved in a partially closed system using ozone compared to identical systems using oxygen and air (Ajuzie and Appelbaum, 1993).

The use of ozone in recirculation systems shows great potential, and many companies are reporting some success in controlling the various problems outlined above. Undoubtedly ozonation of water will play an increasing role in future recirculating aquaculture systems.

6.4.7 ION EXCHANGE MEDIA

Various resins have been developed which exchange cations and anions with those in the water. These include synthetic resins and naturally occurring minerals such as zeolite and kaolins. The advantage of these materials is that they can be recharged by soaking in bleach, dechlorinator and buffer solution. Various

successes have been reported with these compounds although in sea water these compounds are quickly flooded with sodium, calcium, magnesium and chloride ions and are of no practical use. Turner and Bower (1983) found commercial resins to be not as effective as activated carbon whilst Dryden and Weatherley (1989) found clinoptilolite (a type of zeolite) to be irreversibly bound by calcium ions. It would appear that outside the pet market (fresh water aquaria and cat litter) such resins have limited appeal as activated carbon is cheaper and more effective.

6.4.8 DENITRIFYING FILTERS

Water exchange of up to 10% of the system volume is often adopted in recycling systems. This removes the accumulation of nitrate, the end product of the aerobic removal of inorganic nitrogen, from the system. Another reason for this exchange is to remove the brown colour in the water due to organic compounds unavailable for breakdown by heterotrophic bacteria in the biofilter as described above.

In order to make recirculation systems more environmentally sensitive and reduce their water demand, water exchanges should, ideally, be reduced to zero. Ozonation, as discussed, would minimise the requirement to exchange water. In theory, nitrate can be removed by using a denitrifying biofilter although in practice these filters are difficult to manage and may lead to toxic chemicals being released into the recirculating water.

Certain bacteria, under anaerobic conditions, use NO_3 rather than O_2 as the final electron acceptor during metabolism. Nitrate is converted to nitrite, nitrous oxide and eventually nitrogen gas which escapes into the atmosphere. The bacteria must be fed a carbon source which is added as either glucose, lactose methanol or ethanol. If too little carbon is added their chemical reactions are incomplete and an excess of nitrite and harmful substances like hydrogen sulphide may be produced (St. Amant and McCarty, 1969). The best results have been obtained using methanol to maintain a high carbon to nitrogen ratio. If this ratio decreases, much more hazardous compounds such as ammonia, nitrite, hydrogen sulphide and DOC may be produced (Balderston and Sieburth, 1976). Denitrifying filters can therefore be difficult to control and possibly produce more problems than they alleviate.

One of the most natural forms of nitrate removal is practised in the construction of artificial ecosystems and large aquaria such as dolphinariums. This involves algal scrubbing, the methods of which are described in detail by Adey and Loveland (1991). Briefly, the method employs shallow tanks containing highly photosynthetically efficient macroalgae through which the recirculating water is passed. The algae form turfs and are illuminated at night therefore supplying oxygen as well as removing carbon dioxide and nitrate. Illumination periods may be varied as an effective control of oxygen production and nitrate removal. Algae are cropped as a method of permanently removing nitrogen from the system. In theory, algae could be used to feed

herbivorous fish in another part of the aquaculture system.

The system developed in this study provides efficient water treatment and is one of several water treatment technologies that have been applied to aquaculture. Although several systems are now commercially available, very little research has been performed to assess the interactions between environmental water parameters such as temperature and salinity, the potentially stressful effects of maintaining fish at high stocking densities and the effect this may have on growth rates. The present study has demonstrated the importance of understanding these interacting biological parameters in order to maximise growth potential. Although the Stahlermatic system was originally studied by Knosche (1991), this work concentrated on the efficiency of water treatment rather than the optimal environmental growth conditions for the fish.

6.5 CONCLUSION

The future for closed recycling fish farming looks very promising with many predictions of a prosperous future (Lee, 1992; Cripps, 1995; Willoughby, 1995; Dryden, 1995). This is also discussed in chapter one. Although there are many commercial systems now entering the market, all claiming to be successful, the technologies to be adopted in the future are unknown. The Stahlermatic biofilter used in this study performed well and has been found to do so in full industrial fish farming applications (Knosche, 1991). The success of this system can be partly attributed to its unique self aeration, fixed film and activated sludge systems. An additional advantage is that the biofilter did not become blocked as can occur in trickling filters. Alternative systems which the industry is considering, are the traditional and reliable trickling filters. Removal of waste solids (faeces and uneaten food) would appear to be the most efficient way ahead coupled with recycling of the waste perhaps as plant fertilizer. Ozone is expected to play a large part in future designs due to its capacity to breakdown otherwise unavailable large organic compounds responsible for water discolouration and even accumulated toxins. The foam fractionator (protein skimmer), used in this study to remove proteins from the water, worked well, but if ozone were to be used it would not be necessary. Ultraviolet light would appear to be best incorporated into the influent water but does have some uses within the system. Denitrification is essential if the technology is to advance and although

anaerobic denitrification filters are hard to control, the use of algal scrubbers appears to be an environmentally responsible direction to pursue especially if the algal crop can be fed to herbivorous or omnivorous fish or other useful plant crops can be grown.

In conclusion, it would be interesting to investigate the performance of the system used in this study following the addition of a form of ozone treatment, solids removal and nitrate removal by algal scrubbing.

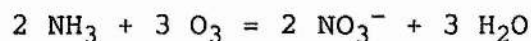
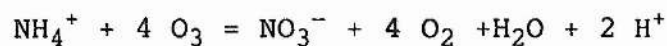
APPENDIX 1

APPENDIX 1

A Summary of Some Chemical Reactions
Utilising Ozone in a Closed
Recirculating Aquaculture System

SUMMARY OF CHEMICAL REACTIONS OF OZONE

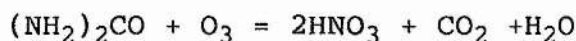
Ammonia:



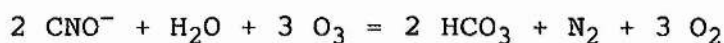
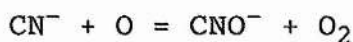
Nitrite:



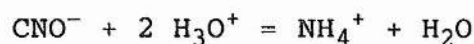
Urea:



Cyanide:



OR



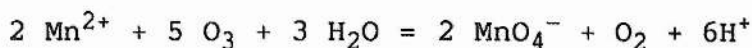
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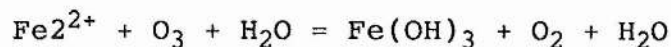
Manganese:



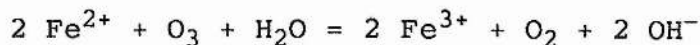
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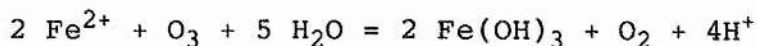
Iron:



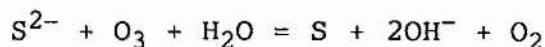
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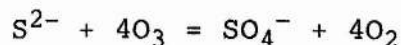
OR



Sulphur:



OR



BIBLIOGRAPHY

RE-CLASSIFICATION OF RAINBOW TROUT

It should be noted that, due to re-classification, rainbow trout is referred to in the text of the thesis as *Oncorhyncus mykiss* but may be referred to in the bibliography as *Salmo gairdneri* which describes the same species.

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